THE ANALYST

MEMBERS of the Society are already aware, from the announcement made at the Annual General Meeting held at the end of February last, and from the Report of the Council for 1955 published in the May issue of *The Analyst*, that the subscription of Ordinary Members will be raised in 1957, and that the cost of *The Analyst* and *Analytical Abstracts* to non-members will also be increased. The subscription of Junior Members will, we are happy to say, remain unchanged.

The Council has taken the step of making these increases only after the most searching enquiries into the cost of running the Society and after long deliberation of the various heads under which our expenditure falls. It will, of course, be realised that the activities of the Society have expanded very widely in the years since 1945. Not only have we extended the size and circulation of *The Analyst*, which now goes to 56 countries and of which we print over 6000 copies a month, but we have taken over from the Bureau of Abstracts the task of providing in *Analytical Abstracts* a monthly journal covering the whole field of analytical chemistry in a manner never before achieved.

While this development has been going on, we have, by the formation of Subject Groups and by increasing the number of our geographical Sections, brought our scientific meetings to a very high number each year, many times that of pre-war days. We have, moreover, enabled analysts in many parts of the country to hear and discuss papers which before our expansion they could only have read.

The increase in the size of *The Analyst*, the launching of *Analytical Abstracts*, and the need to provide office services for the Groups and Sections, together with the work entailed by the very greatly increased membership of the Society, have led to a big expansion of the office staff and to the need to provide proper and centralised offices. These we have found on the top floor of the new house of the Society of Chemical Industry in Belgrave Square, London.

We are a Society that has always been most reluctant to raise its subscription. Few Societies can boast, as we can, that their annual subscription remained unchanged from 1874 to 1950, and at the modest figure of one guinea at that. We have been able to manage in the last few years by no more than doubling that amount. But the need now arises to make a further increase—of no more than another guinea, it is to be noted; and it arises not only from the development of our activities and the necessity for trying to save a little money each year against future expansion, but from those increases in prices that we see around us all today, and especially in the cost of printing.

It would have been necessary to increase the subscription of members and the cost of our publications some years ago had not the Chemical Council made us grants which enabled the Society to balance its publications accounts. These grants were made from funds subscribed by industry for the publication of chemical researches, and the continuing success of our journals prompts us to believe that we have applied them as the donors would have wished. We can never be too grateful for the aid that has thus come to us; equally it seems no more than right that we should use all our endeavours to make *The Analyst* and *Analytical Abstracts* self-supporting, or as nearly so as we can, and at the same time to place the general finances of the Society on a footing that can cope in the foreseeable future with the demands likely to be made on them. It is with these objects in view that the Council of the Society has acted.

K. A. WILLIAMS
President

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

JOINT MEETING

A JOINT Meeting of the Society with the Food Group of the Society of Chemical Industry was held at 6.30 p.m. on Wednesday, May 23rd, 1956, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Food Group, Dr. A. J. Amos, F.R.I.C.

The following papers were presented and discussed: "Some New Factors in Pectin Gel Strength," by Mamie Olliver, M.Sc., F.R.I.C., P. Wade, M.Sc., Ph.D., D.I.C., A.R.I.C., and Kathleen P. Dent, A.R.I.C.; "The Binding of Ions and Detergents to Pectin, Protein and Other Colloid Systems," by B. A. Pethica, B.Sc., Ph.D., A.R.I.C. The discussion was opened by C. L. Hinton, F.R.I.C.

NEW MEMBERS

ORDINARY MEMBERS

Robert Anthony Bastow, B.Sc. (Nottingham), A.R.I.C.; John Edward Butler, A.R.I.C.; James Stark Foster, B.Sc. (Glas.); Margaret Jean Guy, B.Sc. (Liv.), B.Sc. (Lond.); Joseph Hall, B.Sc. (Lond.); Geoffrey Frederick Harrison, B.Sc. (Lond.); Oscar Lazar, B.Sc.Eng.; Thomas Emerson Lonsdale, B.Sc. (St.A.); William Kenneth Matthews, F.R.I.C.; Arthur William McGill, B.Sc. (Lond.), A.R.I.C.; Rowland Frost Roberts; Robert Hogarth Robertson, B.Sc. (Glas.), A.R.I.C.; Ronald Sawyer, B.Sc. (Sheff.); Joan Mary Sillibourne, B.Sc. (Lond.); Arthur Stanley Smith, B.Sc. (Wales); Peter Sutcliffe.

JUNIOR MEMBERS

Leon Erwin Cohen, B.S. (New York); Winifred Jean Graham; Stanley Bernard Kissen, B.Sc. (Glas.).

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Friday, May 11th, 1956, in the George Hotel, George Street, Edinburgh. The Chair was taken by the Chairman of the Section, Dr. F. J. Elliott, F.R.I.C., F.R.S.E.

A lecture on "Complexones: Some Recent Developments" was given by R. E. Stuckey, B.Sc., Ph.D., F.P.S., F.R.I.C.

JOINT COMMITTEE ON METHODS OF ASSAY OF CRUDE DRUGS

A JOINT Committee has been formed by the Pharmaceutical Society and the Society for Analytical Chemistry to prepare standard methods of assay for crude drugs and kindred materials where such methods are required in commerce and are not included in current editions of the British Pharmacopoeia and the British Pharmaceutical Codex. The committee will receive and examine proposals for the preparation of methods and will allocate these, if approved, for detailed investigation to small working panels of experts in the subjects. Methods submitted by the panels and accepted by the committee will be published as standard methods.

The constitution of the committee is as follows:—Dr. K. R. Capper (*Chairman*), Dr. A. J. Feuell, Dr. D. C. Garratt (*ex officio* as Chairman of the Analytical Methods Committee of The Society for Analytical Chemistry), Mr. R. Higson, Mr. C. A. Johnson, Mr. H. C. Macfarlane, Dr. W. Mitchell, Mr. W. M. Seaber, Dr. R. E. Stuckey, Dr. C. H. Tinker (*Secretary*) and Mr. D. Watt.

The Determination of 4-Chloro-2-methylphenoxyacetic Acid in MCPA by a Differential Refractometric Method

By R. HILL

(*Presented at the meeting of the Society on Wednesday, April 4th, 1956*)

A differential refractometric method is described for the determination of the biologically active 4-chloro-2-methylphenoxyacetic acid in MCPA containing 80 to 100 per cent. of the active acid. The method involves the comparison of the refractive index of a saturated solution of pure 4-chloro-2-methylphenoxyacetic acid with that of a solution of the sample prepared in such a way that all the impurities in the sample are dissolved and the solution is saturated with the main component. The difference in refractive index between the two solutions is an accurate measure of the impurity content of the sample, provided that the impurities are methyl- or chloromethyl-phenoxyacetic acids or both. The results for synthetic samples are in good agreement with the actual values and the precision of a single determination is better than ± 1 per cent. expressed as 95 per cent. confidence limits.

Many selective herbicide formulations are based on MCPA, which is a mixture of chloromethylphenoxyacetic acids containing 4-chloro-2-methylphenoxyacetic acid as the active principle together with various proportions of 6-chloro-2-methyl-, 4 : 6-dichloro-2-methyl- and 2-methyl-phenoxyacetic acids.

Several methods for the determination of 4-chloro-2-methylphenoxyacetic acid in such mixtures have been published, including an isotope-dilution method by Sørensen,[1,2] an infra-red spectrophotometric method by Sjöberg,[3] ultra-violet spectrophotometric methods by Grabe[4] and by Hill[5] and a chromatographic method by Freeman and Gardner.[6] The ultra-violet and chromatographic methods, which are the most suitable for routine analyses, have been used extensively in these laboratories, but because the accuracy obtainable with the ultra-violet methods is dependent to a great extent on the composition of the sample, the chromatographic method has generally been preferred. However, even this method has not proved to be entirely satisfactory, because of the considerable time required to train an unskilled operator in the art of column packing and more particularly because of the poor precision of the results, which, expressed as 95 per cent. confidence limits of a single determination, has been found to be ± 3 per cent. in a research laboratory and ± 5 per cent. in a process-control laboratory.

Attempts to improve the precision of the chromatographic method were unsuccessful and in view of the fact that a large proportion of the MCPA produced at the present time contains 80 to 90 per cent. of 4-chloro-2-methylphenoxyacetic acid attention was turned to the possibility of using a differential refractometric method,[7] which it was considered would be capable of a precision of better than ± 1 per cent. This method involves a comparison of the refractive index of a saturated solution of pure standard with that of a solution of the sample prepared in such a way that all the components of the sample are dissolved except a small amount of the major component. The difference in refractive index between the two solutions is dependent upon the refractometric behaviour and amounts of the various impurities in the sample. Provided that the impurities are similar in refractometric behaviour, the difference in refractive index can be used as an accurate measure of the total impurity content.

Experimental

Technique of differential refractometry—

The construction and method of use of a differential refractometer for the determination of the purity of organic compounds has been described by Hill and Jones.[7] No major modification was made to the apparatus described by these authors, but since brass was attacked

by phenoxyacetic acids it was found necessary to construct the three-compartment refractometer cell, which had a 90° central prism, from stainless steel.

It was also found necessary to modify the procedure for preparing saturated solutions, since that described by Hill and Jones for gamma benzene hexachloride was not reproducible when applied to MCPA. The procedure adopted is described subsequently under "Method" (see p. 326).

CHOICE OF SOLVENT—

Ideally the selected solvent should be one such that a given volume will dissolve all the impurities in a relatively large sample and leave a small excess of the main component undissolved. For this to occur it is necessary that the ratio of the amount of main component in the sample to the amount of an individual impurity should be greater than the ratio of the solubility of the main component to that of the individual impurity, for each impurity in the sample, *i.e.*—

$$\frac{100 - x}{y} > \frac{\text{solubility of the main component}}{\text{solubility of individual impurity}},$$

where x is the percentage of total impurity and y is the percentage of the individual impurity.

If the above condition does not hold good for any one impurity, then it is necessary to add some pure main component to the sample in order that the sample solution should be saturated with this.

It was known that 4:6-dichloro-2-methylphenoxyacetic acid, which was a likely impurity in MCPA, was in general much less soluble than the 4-chloro-2-methyl acid, and in consequence the approximate solubilities of these two acids were determined in a number of common solvents with the results given in Table I. The solubilities of 6-chloro-2-methyl- and 2-methylphenoxyacetic acids were not determined at this stage, since it was known that the solubility of the former acid was greater than that of 4-chloro-2-methylphenoxyacetic acid and that of the latter acid was of the same order as the solubility of 4:6-dichloro-2-methylphenoxyacetic acid.

TABLE I

APPROXIMATE SOLUBILITIES OF 4-CHLORO-2-METHYL- AND 4:6-DICHLORO-2-METHYLPHENOXYACETIC ACIDS IN SOME COMMON SOLVENTS

Solvent	Temperature, °C	Solubility of 4-chloro-2-methylphenoxyacetic acid, g per 100 g of solvent	Solubility of 4:6-dichloro-2-methylphenoxyacetic acid, g per 100 g of solvent
Benzene	21	4·2	0·4
Toluene	21	3·7	0·4
n-Butanol	21	56	7·3
*iso*Propanol	21	79	8·9
Chloroform	21	4·2	0·7
n-Butyl acetate ..	15	22	3·4

Considering a maximum impurity content of 20 per cent., all of which could be 4:6-dichloro-2-methylphenoxyacetic acid, it was obvious that none of the solvents examined fulfilled the condition stated above and it would be necessary therefore to add pure 4-chloro-2-methylphenoxyacetic acid to the sample.

The solvent chosen for further work was *n*-butyl acetate, since it possessed a reasonably high solubility for the acids, was not too volatile and was readily available in a sufficiently pure state.

REFRACTOMETRIC BEHAVIOUR OF A NUMBER OF SUBSTITUTED PHENOXYACETIC ACIDS IN *n*-BUTYL ACETATE SOLUTION—

With *n*-butyl acetate as solvent, 0·5 per cent. w/v solutions of fifteen phenoxyacetic acids were prepared and compared with pure solvent in the differential refractometer, the linear shift of the slit image, Δd, being measured for each solution. In addition, the relation between Δd value and concentration was investigated for solutions of 2-methyl-, 6-chloro-2-methyl- and 4:6-dichloro-2-methyl-phenoxyacetic acids in *n*-butyl acetate. The results of these two series of experiments, which are recorded in Tables II and III, showed that the differences in refractometric behaviour between mono-, di- and tri-substituted phenoxyacetic

acids were relatively small and that the relation between Δd and concentration was linear over the range 0 to 2 per cent. w/v.

TABLE II

REFRACTOMETRIC BEHAVIOUR OF A NUMBER OF PHENOXYACETIC ACIDS

Phenoxyacetic acid	Δd for 0·5 per cent. w/v solution in *n*-butyl acetate, cm
Unsubstituted	0·125
2-methyl	0·125
3-methyl	0·124
2-chloro	0·125
4-chloro	0·123
2:4-dichloro	0·118
2:6-dichloro	0·118
4-chloro-2-methyl	0·120
6-chloro-2-methyl	0·122
4-chloro-3-methyl	0·118
2-chloro-4-methyl	0·118
4:6-dichloro-2-methyl ..	0·116
2:6-dichloro-4-methyl ..	0·106
2:4:6-trichloro	0·109
2:4:5-trichloro	0·111

TABLE III

RELATION OF CONCENTRATION TO Δd FOR SOLUTIONS OF 2-METHYL-, 6-CHLORO-2-METHYL- AND 4:6-DICHLORO-2-METHYL-PHENOXYACETIC ACIDS

Concentration of acid, % w/v	Δd for 2-methylphenoxyacetic acid, cm	Δd for 6-chloro-2-methylphenoxyacetic acid, cm	Δd for 4:6-dichloro-2-methylphenoxyacetic acid, cm
0·5	0·125	0·122	0·116
1·0	0·252	0·242	0·230
1·5	0·375	0·362	0·348
2·0	—	0·484	0·468

It was apparent therefore that for calibration purposes the nature of the impurity used was not critical.

PREPARATION OF PURE 4-CHLORO-2-METHYLPHENOXYACETIC ACID—

Pure 4-chloro-2-methylphenoxyacetic acid, m.p. 120·15° to 120·2° C, was prepared by the method described by Sjöberg.[3] This method gave a consistently pure product, but was somewhat time-consuming and the possibility of preparing pure 4-chloro-2-methylphenoxyacetic acid from commercial MCPA containing about 90 per cent. of this acid was investigated. Although no completely satisfactory method was found, the following route usually led to material of 99·7 to 99·9 per cent. purity, m.p. 120·0° to 120·1° C, which was suitable for use as a secondary standard. About 1 kg of commercial MCPA was dissolved in about 1·5 litres of cold industrial methylated spirit. An equal volume of water was then added slowly and with stirring to precipitate most of the dissolved acids. The precipitate was removed by filtration, dried at 105° C and crystallised successively from acetone, benzene and chloroform. The yield on starting material was about 30 per cent.

DEVELOPMENT OF METHOD—

It was proposed that 2·5 g of sample mixed with 2·0 g of pure 4-chloro-2-methylphenoxyacetic acid should be extracted at 15° C with 20 ml of *n*-butyl acetate and the resulting solution compared in a differential refractometer with a saturated solution of 4-chloro-2-methylphenoxyacetic acid prepared under the same conditions. By this procedure it was considered that 0·2 per cent. of impurity should be determinable, since this would give a

Δd value of about 0·004 cm (see Table II), which could be measured with a reasonable degree of accuracy with the instrument available.

Very satisfactory results were obtained by the above procedure for artificial mixtures containing 80 to 100 per cent. of 4-chloro-2-methylphenoxyacetic acid and also for a few commercial MCPA samples. The majority of commercial samples could not be analysed, however, because of the presence in them of coloured impurities that absorbed a considerable amount of the light entering the refractometer cell and made observation of the slit image in the microscope eyepiece extremely difficult if not impossible.

It was found that this difficulty could be overcome by reducing the weight of sample taken to 1 g and increasing the weight of pure 4-chloro-2-methylphenoxyacetic acid to 3·5 g. This involved the use of large quantities of the 4-chloro acid, which was difficult to prepare in a pure state, and so the possibility of extracting the sample with *n*-butyl acetate mixed with some solvent of about the same boiling point in which the phenoxyacetic acids are more or less insoluble was investigated.

It was found that 20 ml of a (60+40 v/v) mixture of *n*-butyl acetate and "*iso*octane" (2:2:4-trimethylpentane), in which the approximate solubilities of the 4-chloro-2-methyl-, 4:6-dichloro-2-methyl and 2-methyl-phenoxyacetic acids at 15° C were 10·0, 1·2 and 1·0 per cent. w/w, respectively, would conveniently extract 1·0 g of sample mixed with about 1·5 g of pure 4-chloro-2-methylphenoxyacetic acid. With this system up to 19 per cent. out of a total impurity content of 20 per cent. could be tolerated for 4:6-dichloro-2-methylphenoxyacetic acid and up to 16 per cent. for the 2-methyl acid.

Method

Apparatus—

Differential refractometer—A non-recording instrument, with which differences in refractive index of 10^{-5} units or less can be detected.[7] The refractometer cell should be of stainless steel or some other material unaffected by phenoxyacetic acids.

A thermostatically controlled water bath capable of operating below room temperature and able to maintain a desired temperature to within $\pm$ 0·05° C.

Flat-bottomed flasks of 50 ml capacity fitted with B19 sockets and equipped with stoppers carrying stirrers.

Reagents—

Solvent—A (60 + 40 v/v) mixture of *n*-butyl acetate and "*iso*octane" (2:2:4-trimethylpentane). Commercial grade solvents may be used provided that they are free from coloured impurities.

4-Chloro-2-methylphenoxyacetic acid, pure, m.p. 120·1° *to* 120·2° C.
6-Chloro-2-methylphenoxyacetic acid, pure, m.p. 109° *to* 110° C.
4:6-Dichloro-2-methylphenoxyacetic acid, pure, m.p. 187° *to* 188° C.
2-Methylphenoxyacetic acid, pure, m.p. 154° *to* 155° C.
Sulphuric acid, 10 *per cent. v/v.*
Sodium bicarbonate—A half-saturated aqueous solution.
Sodium sulphate, anhydrous.
Sodium hydroxide, 0·1 N.
Ether—Analytical-reagent grade.
Chloroform—Analytical-reagent grade or the B.P. grade.
Phenolphthalein—A 0·2 per cent. solution in 50 per cent. v/v aqueous ethanol.

Procedure for commercial MCPA—

The procedure described below refers to the commercial acid as distinct from formulations containing MCPA. However, it can be adapted quite readily to formulations such as aqueous solutions of amine and alkali-metal salts of MCPA and, after preliminary hydrolysis, to esters also.

Commercial MCPA, which is sold in granular or flake form, is likely to contain traces of chlorocresols and sodium chloride together with up to 10 per cent. of water, all of which interfere in the differential refractometric examination and must be removed beforehand. This means that a determination of the total chloromethylphenoxyacetic acid content of the sample is required in order to evaluate the amount of 4-chloro-2-methylphenoxyacetic acid originally present.

DETERMINATION OF TOTAL MCPA CONTENT OF SAMPLE—

Weigh out accurately sufficient sample to contain 0·5 to 1·0 g of the mixed chloromethylphenoxyacetic acids and dissolve it in 50 ml of chloroform in a separating funnel. Extract the chloroform solution with one 25-ml and two 10-ml portions of half-saturated sodium bicarbonate solution and combine the extracts. Acidify them carefully with dilute sulphuric acid and extract the liberated acids, free from chlorocresols, with three portions of chloroform (25, 20 and 15 ml). Combine the chloroform extracts and wash the bulk with 10 ml of distilled water. Transfer the chloroform layer to a 250-ml conical flask, extract the aqueous layer with 5 ml of chloroform and run the latter into the flask. Boil off almost all the chloroform by heating on a steam-bath, dissolve the residue in 20 ml of neutral ethanol and titrate with 0·1 *N* sodium hydroxide, using phenolphthalein as indicator. Determine the equivalent weight of the extracted acids by titrating about 0·5 g of the specimen obtained for differential refractometric examination (see below) with 0·1 *N* sodium hydroxide as described above. Calculate the percentage of chloromethylphenoxyacetic acids from the weight of sample taken, the titre obtained and the equivalent weight.

EXTRACTION OF A SUITABLE SPECIMEN OF MCPA FOR DIFFERENTIAL REFRACTOMETRIC EXAMINATION—

Take about 5 g of the sample and dissolve it in 100 ml of ether in a separating funnel. Extract the ether layer with three 50-ml portions of half-saturated sodium bicarbonate solution. Acidify the combined bicarbonate extracts carefully with sulphuric acid (beware of frothing) and extract the liberated acids three times with 50 ml of ether. Dry the combined ether extracts over anhydrous sodium sulphate, transfer the dried ether solution to a 800-ml beaker and carefully evaporate to dryness. When dry, break up the solid into small pieces, transfer it to a smaller vessel and dry it at 80° to 100° C for ½ hour. Grind the product finely and store it in a stoppered bottle until required.

DIFFERENTIAL REFRACTOMETRIC EXAMINATION OF THE ISOLATED SPECIMEN—

Weigh out 1·0 g of the extracted MCPA together with 1·3 to 1·5 g of pure 4-chloro-2-methylphenoxyacetic acid into a 50-ml flat-bottomed flask and 3·2 g of pure 4-chloro-2-methylphenoxyacetic acid into a second similar flask. Add 20·0 ml of *n*-butyl acetate - "*iso*octane" solvent to the first flask and 30·0 ml to the second and insert stoppers and stirrers. Support the flasks in a water bath at 15° C or any other convenient temperature between 15° C and 2° C below room temperature and stir the mixture mechanically for 2 to 3 hours. Allow the contents of the flasks to settle, withdraw the supernatant solutions through cotton-wool filters into suitable pipettes and transfer to clean dry stoppered 2-oz bottles. Set the solutions aside for 1 hour to attain room temperature.

Ten minutes before the instrument is required switch on the mercury lamp of the differential refractometer and fill the tank surrounding the cell with water at room temperature.

Clean the refractometer cell by washing it with acetone, dry it by means of a current of air and place it securely in position in the water bath.

With a clean dry pipette fill each of the cell compartments with the solution of pure standard and replace the caps on the outlet tubes of the compartments, leaving the cap of the centre one quite loose.

Switch on the water-bath stirrer and allow 10 minutes for the cell to reach the temperature of the water bath.

Adjust the variable slit in the instrument to a convenient width and align the travelling microscope cross-wires with the image of the slit, making a fine adjustment of the focus if necessary; it is imperative that the optical distance from eye-piece to refractometer cell should not vary by more than ± 1 cm from the distance employed for the preparation of the calibration curve. Read the vernier scale to the nearest 0·001 cm to obtain the zero reading.

Without removing the refractometer cell from the water bath, unscrew the cap of the centre compartment and carefully withdraw its contents with a pipette. Wash out the centre compartment with three successive small portions of the sample solution, fill with sample solution and replace the cap.

Allow 10 minutes for the cell and its contents to reach temperature equilibrium with the stirred water in the bath, re-align slit image and microscope cross-wires, without making focus adjustment, and read the vernier scale to obtain the sample reading.

Calculate the difference between the sample and zero vernier readings to obtain Δd and read off the 4-chloro-2-methylphenoxyacetic acid content of the extracted MCPA from a calibration graph prepared as described below. From the value so obtained and the total chloromethylphenoxyacetic acid content of the sample determined above, calculate the percentage of 4-chloro-2-methylphenoxyacetic acid in the sample.

PREPARATION OF CALIBRATION GRAPH—

Repeat the above determination of Δd for artificial mixtures containing 4·0, 8·0, 12·0, 16·0 and 20·0 per cent. of impurity, using a mixture of equal parts of 6-chloro-2-methylphenoxyacetic acid and 4:6-dichloro-2-methylphenoxyacetic acid as the impurity. Prepare the calibration graph by plotting Δd against percentage of 4-chloro-2-methylphenoxyacetic acid.

This graph is suitable for the analysis of MCPA samples having equivalent weights between 200 and 205. For samples with equivalent weights between 195 and 200 a fresh series of artificial mixtures in which a mixture of equal parts of 6-chloro-2-methyl- and 2-methyl-phenoxyacetic acids are used as impurity should be examined.

RESULTS AND DISCUSSION

A series of artificial mixtures whose compositions were unknown to the analyst was examined, with the results shown in Table IV.

TABLE IV

ANALYSIS OF ARTIFICIAL MIXTURES CONTAINING 80 TO 100 PER CENT. OF 4-CHLORO-2-METHYLPHENOXYACETIC ACID

Composition of substituted phenoxyacetic acid mixture				
4:6-Dichloro-2-methyl, %	6-Chloro-2-methyl, %	2-Methyl, %	4-Chloro-2-methyl, %	4-Chloro-2-methylphenoxyacetic acid found, %
5·0	5·0	0·0	90·0	89·7
8·3	4·5	2·1	85·1	85·2
10·1	2·0	0·5	87·4	87·7
16·0	4·0	0·0	80·0	80·2
5·1	0·0	0·0	94·9	94·9
7·5	2·5	0·0	90·0	90·1
7·0	5·1	4·2	83·7	83·9
9·7	0·8	1·4	88·1	88·0
0·0	10·0	0·7	89·3	89·1
2·5	0·0	0·0	97·5	97·5

All these results were within 0·3 per cent. of the actual values and this order of accuracy was considered to be satisfactory in view of the large impurity range covered,

The precision or repeatability of the method was assessed from the results of duplicate analyses of 43 MCPA samples carried out in the Research Department by experienced operators. The results were used to calculate the standard deviation, S^2, of a single determination by means of the following expression—

$$S^2 = \frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2 + \ldots + \Sigma(x_n - \bar{x}_n)^2}{(N_1 - 1) + (N_2 - 1) + \ldots + (N_n - 1)},$$

$\bar{x}_1, \bar{x}_2 \ldots \bar{x}_n$ are the mean values for samples, 1, 2 . . . n,
$x_1, x_2 \ldots x_n$ are the values for individual determinations in samples 1, 2 . . . n, and
$N_1, N_2 \ldots N_n$ are the number of determinations made for samples 1, 2 . . . n.

From this value the 95 per cent. confidence limits, *i.e.*, the range in which 19 out of 20 results of single determinations would be expected to lie, were calculated and found to be $\pm$ 0·7 per cent.

A further series of results obtained under routine conditions by operators with limited experience of the method is given in Table V. The 95 per cent. confidence limits for these results were $\pm$ 0·8 per cent.

It was obvious that the results were much more precise than those obtained by any other published method, but there was some doubt about the effect on the accuracy of the method of coloured tarry materials, 1 to 2 per cent. often being present in MCPA.

To check the effect of this tarry material, which was obtained as a residue by vacuum-sublimation of a sample of MCPA, an artificial sample was prepared containing 94·0 per cent. of 4-chloro-2-methylphenoxyacetic acid with a mixture of equal parts of the tar, 6-chloro-2-methyl- and 4 : 6-dichloro-2-methyl-phenoxyacetic acids as impurity, and this was analysed five times. The average value obtained for the 4-chloro-2-methylphenoxyacetic acid content of the sample was 93·8 per cent., which was not significantly lower than the actual value. It was considered therefore that any systematic error resulting from the presence of tarry impurities would be small compared with the random errors and the effect could be neglected.

TABLE V

REPLICATE ANALYSES OF EXTRACTED ACIDS FROM SOME COMMERCIAL MCPA SAMPLES PERFORMED UNDER ROUTINE CONDITIONS

Individual values for 4-chloro-2-methylphenoxyacetic acid, %			Average, %
81·9	81·3	82·2	81·8
82·2	82·6	83·0	82·6
87·3	87·1	86·7	87·0
81·9	82·2	82·6	82·2
85·2	85·5	84·9	85·2
85·3	85·7	85·0	85·3
86·5	86·0	86·3	86·3
82·7	82·8	82·8	82·8
88·1	88·4	—	88·3
81·2	81·3	—	81·3
89·0	88·9	—	89·0
89·2	89·5	—	89·4

CONCLUSIONS

The method described provides a precise and accurate method for the routine determination of 4-chloro-2-methylphenoxyacetic acid in MCPA formulations, the extracted acids from which contain more than 80 per cent. of the 4-chloro-2-methyl acid.

With suitable adjustment of the weight of extracted chloromethylphenoxyacetic acids taken and the composition of the solvent, it should be possible to apply the method to MCPA containing 60 to 80 per cent. of 4-chloro-2-methylphenoxyacetic acid.

The total time required for the analysis of a single sample is 3 to 4 hours, but by examining several samples concurrently it is possible for one operator to carry out up to six determinations in a day.

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The Determination and Distribution of Lead in Human Tissues and Excreta

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A method was described in 1935 for the determination of lead in biological materials. Organic matter was destroyed by ignition, and lead was separated as the diethyldithiocarbamate in ether and finally measured colorimetrically with dithizone. This paper includes descriptions of subsequent modifications and particularly the use of the reversion procedure in the final colorimetric determination.

Results are given to illustrate the distribution of lead in human tissues and excreta for normal subjects and in cases of plumbism and also the effect of medication and such like in the latter condition.

THIS paper is a survey of work carried out in the Biochemical Laboratory, Royal Infirmary, Glasgow, during the period 1933 to 1946, and so references are mainly restricted to work published from this laboratory. During this period, patients suspected to be suffering from lead poisoning were frequently admitted to hospital for diagnosis and treatment. Plumbism was mainly the result of industrial exposure, but in a few cases contaminated water supplies were suspected. The investigations were made for two purposes: (*a*) to devise a satisfactory procedure for the determination of lead in a wide range of biological materials, *e.g.*, urine, faeces, soft tissues, bone, blood and so on, and (*b*) to determine satisfactory clinical methods for the assessment of plumbism and its control during treatment.

THE DETERMINATION OF LEAD

The method published in 1935[1] has in general been modified but slightly and this mainly in manipulative details. Large samples of materials were taken for analysis, since: (*a*) the ranges of concentrations of lead to be determined in many of these materials were uncertain, (*b*) to ensure that the blank represented only a small fraction of the lead to be finally determined, and (*c*) to ensure a more accurate average value, as sampling in many instances was difficult.

The procedure readily fits into the routine work of a hospital biochemical laboratory and is applicable to the biological materials, many differing greatly in composition, requiring analysis in the examination of plumbism. At certain stages, analysis may be discontinued without detriment to the final result. The procedure can be resolved into three stages, which are treated in the three sections that follow.

DESTRUCTION OF ORGANIC MATTER—

Organic matter was destroyed by ignition in a silica dish over a bunsen burner in a fume cupboard. With materials of low ash content, *e.g.*, blood and soft tissues, sodium phosphate was added before ashing. Sodium phosphate solutions can be readily freed from lead. No addition was made to urine, faeces or bone.

Lead added to any of these materials and subjected to the complete procedure, including the ashing process, could be determined quantitatively.

SEPARATION AND CONCENTRATION OF THE LEAD—

Before colorimetric determination of the lead, it is necessary to effect its separation. Separation as the sulphide was the usual procedure until Allport and Skrimshire[2] and Lynch, Slater and Osler[3] suggested the extraction of lead as the dithizonate with chloroform. I encountered difficulties with this method when it was applied to solutions of the ash of certain materials. This appeared to be due in part to the high iron content of these materials and the ease with which dithizone is oxidised to a yellow inactive compound. This aspect of the problem was not investigated further, since an alternative procedure presented itself.

Sodium diethyldithiocarbamate had recently been introduced by Callan and Henderson[4] for the colorimetric determination of copper. It was found that this substance, relatively

more stable than dithizone, formed a compound with lead that was easily soluble in ether, the extracts being colourless. Extractions were carried out at pH 7·5 to 8·0 in the presence of excess of citrate to prevent the co-extraction of iron and the precipitation of the phosphates of the alkaline earths. Under these conditions, copper is also extracted, and so the extracts are coloured yellow. Later,[5] potassium cyanide was added before the formation of the heavy-metal diethyldithiocarbamates. Under these conditions, copper diethyldithiocarbamate is not formed, and hence ether extracts should be colourless. This is a useful index to indicate that traces of iron are not being co-extracted.

Traces of iron tend to interfere with the colorimetric determination of lead with dithizone. "Accidental" co-extraction of iron is indicated by the ether extract assuming a dirty brown colour. In such rare cases, ether extracts must be collected, the ether removed by evaporation and organic matter destroyed. Extraction as lead diethyldithiocarbamate should then be repeated.

Since there was little information available about the stability of sodium diethyldithiocarbamate or its heavy-metal compounds, the reagent was added after the first ether addition, and extraction and separation was carried out without delay.

Extractions and separations can be made either in a separating funnel or in a glass-stoppered cylinder with the aid of a pipette. I prefer use of the latter apparatus, since contamination of the ether extract with the aqueous phase is reduced.

After evaporation of the ether, organic matter in the residue was destroyed by heating it with sulphuric and perchloric acids. This acid mixture has been replaced by 100-volume hydrogen peroxide, since the use of perchloric acid in this procedure has resulted in an accident involving the loss of an eye.

A modification was later introduced[6] for use with materials such as bone and faeces, which contain high concentrations of the alkaline-earth phosphates. With these materials difficulties may arise because of the precipitation of insoluble phosphates in alkaline solutions, even in the presence of excess of citrate. A preliminary extraction of lead as the diethyldithiocarbamate with ether from an acid solution (0·5 *N* hydrochloric acid) was made. Ether extracts were separated, the ether was removed by evaporation and organic matter was then destroyed. Lead was then re-extracted as the diethyldithiocarbamate with ether from an alkaline solution in the presence of citrate and cyanide. Many other metals are extracted with ether as diethyldithiocarbamate when the procedure is carried out in an acid solution. The extracts are coloured a dirty brown owing to the predominance of iron. This may be used as a useful index with respect to complete extraction. It was impossible to calculate whether excess of sodium diethyldithiocarbamate had been added to cope with all the reactive metals present. Additional reagent was therefore added after each ether addition, the colour of the extract being used as an index. This procedure was examined by measuring the recovery of lead added to faeces or milk.

COLORIMETRIC DETERMINATION OF THE LEAD—

It had been the usual practice to determine lead in biological materials colorimetrically by the sulphide reaction. Fischer and Leopoldi[7] suggested that microgram quantities of lead could be determined colorimetrically with dithizone. Anderson and the author[1] successfully applied this reaction to the colorimetric determination of lead in extracts obtained from biological materials. The colour was developed in carbon tetrachloride by using a slight excess of dithizone. The aqueous solution contained ammonium acetate and cyanide and was alkaline with ammonia. Excess of dithizone was removed by extraction of the organic phase with 1 per cent. potassium cyanide solution. The intensity of the pink colour was read against a comparable standard in a visual colorimeter. Although lacking in precision, the visual colorimeter has certain advantages over photo-electric instruments. "Off" colours caused by oxidation products of dithizone could readily be detected. Low concentrations were, however, difficult to measure. Blanks were determined by adding a known amount of lead and calculating by difference after reading against a standard.

Unless precautions are taken, difficulties may be encountered when lead is determined colorimetrically with dithizone. Commercial dithizone contains an inactive yellow oxidation product, which may interfere. I use a freshly prepared aqueous ammoniacal extract of a carbon tetrachloride solution of commercial dithizone. Such an extract is free from the undesirable oxidation product.

Dithizone is readily oxidised to the yellow oxidation product. Precautions must

therefore be taken to prevent its formation during the colorimetric determination of lead with dithizone. The following precautions appear to be necessary—

(*i*) iron salts must be absent—this can be ensured by suitable preliminary extraction procedures;

(*ii*) absence of oxidants—this can be ensured by the addition of a reducing agent, *e.g.*, sulphurous acid; and

(*iii*) the reaction should not be carried out in bright sunlight.

It is believed that in the presence of bright sunlight the ultra-violet component liberates free chlorine produced by the decomposition of carbon tetrachloride.

Before colorimetric analysis, clarification of the extracts is necessary. Clearing by centrifuging appears to be the safest procedure. Filtration through paper appears to have its difficulties. Unwashed paper contributes traces of reactive metals, whereas the acid retained in washed papers tends to produce some reversion if the solution contains the pink lead dithizonate.

Irving, Risdon and Andrew[8] have introduced the principle of reversion into the use of dithizone in the measurement of microgram quantities of metals. Irving and Butler[9] have recently described a method for the determination of lead in biological materials in which this principle is incorporated.

I now use a photo-electric instrument (Unicam SP350 spectrophotometer) for the assessment of the lead - dithizone reaction. Assessment is made by reversion, which is carried out with 0·1 *N* sulphuric acid. Absorptions are read both before and after reversion at 525 mμ (maximum for lead dithizonate) and 620 mμ (maximum for free dithizone). It has been found that the difference of absorption recorded before and after reversion followed Beer's law within the range 0 to 20 μg of lead (10 ml of carbon tetrachloride).

Much trouble can be avoided by completing the procedure with the minimum of delay.

BISMUTH—

Bismuth, if present, is extracted together with lead and reacts with dithizone to produce an orange-coloured solution in carbon tetrachloride. Its presence is easily detectable during the development of the dithizone reaction and is recognisable as an "off" colour by visual colorimetry. In carbon tetrachloride solution it may be removed by repeated extraction with aqueous 1 per cent. potassium cyanide solution. Lead dithizonate is more stable, but is partly removed by such treatment and as a result a comparable standard should be subjected to the same number of extractions. This property, however, is inapplicable to certain methods now in general use.

Methods[10,11] have been published in which the presence of bismuth in the final extract is eliminated.

I have described[12] a method for the determination of bismuth in biological materials. Bismuth was concentrated by the sodium diethyldithiocarbamate - ether technique in the same manner as lead. It was then determined colorimetrically with thiourea. The colorimetric procedure is simple to carry out and although of low sensitivity is relatively specific. The use of the concentration procedure does permit the determination of low concentrations of bismuth in biological materials. The procedure was devised in order to study the distribution of bismuth in human tissues and excreta.

Bismuth is used therapeutically. It may be a component of so called "stomach powders" or given as an injection in the form of the colloidal metal or oxychloride. In the former, absorption from the alimentary tract is minute, but faeces would be heavily contaminated. In such a case the obvious procedure is to collect the sample when such therapy has been discontinued and the alimentary tract cleared of bismuth compounds. In the latter, this form of therapy is restricted to certain types of diseases.

I have examined a wide range of tissues and excreta for the presence of bismuth. Bismuth was detected and measured in the urine and faeces of a young woman who had a history of treatment by injection with colloidal metallic bismuth. On the other hand, in many controls and cases suspected of plumbism, bismuth was not detectable in excreta or tissues.

It does appear, therefore, that tests to show that bismuth is absent are sufficient in most analyses. I now make use of the two following criteria—

(*a*) colorimetric determination (Unicam SP350 spectrophotometer)—correlation between the reversion readings taken at 525 mμ and 620 mμ, and

(*b*) an aliquot of the final lead extract is acidified to a concentration of 10 per cent. with sulphuric acid and 10 per cent. thiourea solution is added. Presence of bismuth is indicated by the formation of a yellow colour.

In the examinations of lead in foodstuffs, Lockwood[13] has also suggested the use of criterion (*b*).

In the event of bismuth being found, an estimate would be made by the thiourea reaction and its exact significance in the dithizone reaction determined.

I consider that if bismuth is present in human tissues and excreta under examination, its detection and determination should be made, since its presence could be of significance.

Method

Reagents—

Sodium phosphate solution, lead-free—A 10 per cent. w/v solution of $Na_2HPO_4.12H_2O$ in water. Before use, add a small quantity of sodium diethyldithiocarbamate (sufficient to cover the point of a knife blade) to about 150 ml of the phosphate solution and then extract it once with 50 ml of ether.

Sodium citrate solution, lead-free—A 20 per cent. w/v solution in water. Store the solution over a 0·1 per cent. solution of dithizone in chloroform. Shake the solution and filter it before use.

Sodium diethyldithiocarbamate solution—A 2 per cent. w/v solution in water, prepared freshly before use.

Potassium cyanide, 1 per cent. solution—Prepared freshly before use.

Potassium cyanide, 10 per cent. solution—Prepared freshly before use.

Standard lead solution, 1 ml ≡ 1 mg of lead—Dissolve 0·1831 g of lead acetate, $Pb(C_2H_3O_2)_2.3H_2O$, in distilled water containing 5 ml of glacial acetic acid and dilute to 1 litre with water.

Ammoniacal ammonium acetate solution—Add 1 ml of concentrated sulphuric acid, 1 ml of glacial acetic acid and 5 ml of ammonia solution, sp.gr. 0·880, to water and dilute to 25 ml. Prepare this solution freshly before use.

Dithizone reagent—Shake 5 ml of 0·1 per cent. solution of dithizone in carbon tetrachloride with 10 ml of dilute ammonia solution (0·5 ml of ammonia solution, sp.gr. 0·880, diluted to 100 ml with water). Spin the mixture in a centrifuge and use the supernatant liquid.

Sulphurous acid, 5 per cent. w/v.

Hydrogen peroxide, 100-volume.

Distilled water—Prepared in an all-glass still.

The following reagents should all be of recognised analytical grade—

Nitric acid, concentrated.
Sulphuric acid, concentrated.
Hydrochloric acid, concentrated.
Acetic acid, glacial.
Ammonia solution, sp.gr. 0·880.
Carbon tetrachloride.

Apparatus—

Glassware should be Pyrex whenever possible.

Silica dishes (4½ inches in diameter) should be cleaned with hot dilute hydrochloric acid before use.

Filter-paper should be washed with dilute hydrochloric acid and then with water.

Procedure for destroying organic matter and extracting lead—

Urine—Evaporate 250 ml of urine in a silica dish to dryness in a hot-air oven (100° C) or on a steam-bath. Destroy organic matter by heating the dish over a bunsen burner in a fume cupboard. Final traces of carbon may be removed by allowing the dish to cool, moistening the ash with nitric acid and re-heating. Dissolve the ash in 75 ml of water containing 5 ml of concentrated hydrochloric acid, using heat to assist dissolution. Filter the solution into a 250-ml glass-stoppered measuring cylinder, using a further 25 ml of water for washing. Add 50 ml of 20 per cent. sodium citrate solution and adjust the pH to between 7·5 and 8·0 by adding ammonia solution. After cooling the solution, add 5 ml of 10 per cent. potassium cyanide. Extract the mixture three times with 50-ml quantities of ether,

adding 5 ml of 2 per cent sodium diethyldithiocarbamate solution after the first ether addition. Extraction involves shaking for 2 minutes. Remove the ether extract without delay with a pipette. Wash the combined ether extracts once with 10 ml of water and then evaporate to dryness in a round-bottomed digestion flask. Destroy organic matter by heating with 1 ml of concentrated sulphuric acid and 1 ml of 100-volume hydrogen peroxide.

Soft tissues (*liver, brain, kidney, etc.*)—Cut the tissue into small pieces with stainless-steel scissors and homogenise a 100-g portion with 100 ml of water. Transfer the emulsion to a silica dish containing 100 ml of lead-free 10 per cent. sodium phosphate solution. The procedure is then exactly as described for urine.

Blood—Collect 20 ml of blood in an all-glass syringe fitted with a stainless-steel needle, and place it in a Pyrex-glass tube fitted with a glass stopper. Use of anti-coagulants should be avoided, since there is risk of contamination.

Transfer the blood to a silica dish containing 100 ml of lead-free 10 per cent. sodium phosphate solution. The procedure is then as described above, except that in the final digestion only 0·4 ml of concentrated sulphuric acid is used.

Bone—The concentration of lead depends on the type of bone and also the location from which the sample is selected. Very few laboratories have the facilities to reduce bone to a sufficiently fine state to effect accurate sampling of small quantities. I select samples as follows—

Femur or tibia, a 20-g cross-section from the centre of the shaft.

Rib, a "length" weighing approximately 20 g.

Vertebra, a cross-section weighing approximately 20 g. Bone, selected as above and weighing approximately 20 g, should be ashed in a silica dish.

With the aid of heat, dissolve the ash in sufficient *N* hydrochloric acid to effect solution. Dilute the solution to a convenient volume with water.

Transfer 25 ml of the acid solution to a 100-ml glass-stoppered cylinder and dilute to 50 ml with water. Extract the solution three times with 25-ml quantities of ether, adding 10 ml of 2 per cent. sodium diethyldithiocarbamate solution after each ether addition. At each extraction shake the mixture for 2 minutes and separate the organic phase by means of a pipette without delay. Collect the ether extracts in a Pyrex-glass round-bottomed flask. Remove the ether by evaporation and destroy the organic matter in the residue by heating it with 1 ml of concentrated sulphuric acid and 1 ml of 100-volume hydrogen peroxide.

The colour of the ether extracts may be used as an index to show that sufficient sodium diethyldithiocarbamate has been added to combine effectively with all the reactive metals present.

Heat the residue with 1 ml of concentrated hydrochloric acid and about 50 ml of water to effect solution. Repeat the diethyldithiocarbamate - ether procedure, but exactly as described for urine, *i.e.*, in the presence of citrate and cyanide at pH 7·5 to 8·0.

Faeces—The preliminary treatment depends upon the form of the final result.

A. If the final result is to be expressed in terms of mg of lead per 100 g of dried faeces, then dry the sample on a steam-bath, grind it with pestle and mortar and ash a 10-g sample in a silica dish.

B. If the average excretion over a period of days is to be determined, then homogenise the complete sample with water to a convenient volume. Put 200 ml of the sample in a silica dish, heat it to dryness and ash it.

Then proceed as described for bone.

Diets—It may be necessary to establish the daily intake of lead. Analysis of individual foodstuffs is not necessary. A corresponding diet received in the laboratory should be homogenised with water to a convenient volume. The final volume depends upon the nature of the diet, but varies between 1 and 3 litres for a 1-day diet.

A suitable aliquot is then taken and examined as described under urine.

PROCEDURE FOR COLORIMETRIC DETERMINATION—

With the exception of blood, dilute the digests with water and add the following reagents in order: 1 ml of glacial acetic acid, 5 ml of ammonia solution, sp.gr. 0·880, and water to 25 ml.

Dilute the digest from blood to a volume of 10 ml containing 0·4 ml of glacial acetic acid and 2 ml of ammonia solution, sp.gr. 0·880.

Colorimetric analysis should now be completed as quickly as possible.

Standards—Dilute solutions containing 5, 10 and 20 μg of lead (as acetate) to 10 ml with ammoniacal acetate solution. Then add the following reagents in order: 6 drops of 5 per cent. w/v sulphurous acid, 5 ml of 1 per cent. potassium cyanide solution and 10 ml of carbon tetrachloride.

Add dithizone reagent drop by drop with constant shaking until excess is present, as evidenced by the brownish colour of the aqueous phase. After shaking the mixture for 1 minute, spin it in a centrifuge. Read the absorption of the carbon tetrachloride extract against carbon tetrachloride at 525 mμ and 620 mμ, using a Unicam SP350 spectrophotometer with 10-mm cells. Then shake the carbon tetrachloride extract with 5 ml of 0·1 *N* sulphuric acid. Spin the extracts in a centrifuge and again take readings at 525 mμ and 620 mμ. The difference between the readings taken before and after reversion are indicative of the quantity of lead present.

Carry out a blank at the same time, using 10 ml of ammoniacal acetate reagent.

Unknown—Dilute a quantity of digest containing up to 20 μg of lead to 10 ml with ammoniacal ammonium acetate solution. Sometimes this may necessitate a preliminary trial. Then proceed as described for the standards.

BLANK DETERMINATION—

A complete blank should be carried out on all procedures.

TABLE I

LEAD CONTENT OF "NORMAL" HUMAN TISSUES

	Lead content, mg per kg of fresh tissue
Soft tissues—	
Liver	0·9 to 4·6
Kidney	0·7 to 3·7
Brain	0·2 to 0·7
Bones—	
Rib	5·0 to 12·9
Vertebra	2·6 to 14·7
Femur	18·2 to 108
Tibia	15·3 to 96·5

THE DISTRIBUTION OF LEAD IN HUMAN TISSUES AND EXCRETA

We are indebted to Aub, Minot, Fairhall and Reznikoff[14] for much of the earlier work on the metabolism of lead. It was shown that lead was preferentially deposited in the skeleton, and it was suggested that, although skeletal lead exerts no toxic action, conditions might arise to cause the transfer to the soft tissues. Such a transfer could be accompanied by the onset of the symptoms of plumbism. A high calcium diet was shown to cause a preferential deposition of lead in the skeleton, whereas the reverse was produced by a low calcium diet, acidosis and so on.

Kehoe, Thamann and Cholak[15,16,17] have contributed much to the study of the excretion of lead both by normal subjects and in cases of plumbism.

INGESTION OF LEAD—

Under "normal" conditions a small amount of lead enters the body, mainly via the alimentary tract (in food and drinking water). This may amount to 0·2 mg or more per day.[1] An approximate estimate of the intake may be made from an analysis of the faeces. In industrial plumbism, considerable quantities of lead may enter the body through the respiratory tract.

EXCRETION OF LEAD—

Lead is excreted mainly via the alimentary tract (bile, intestinal tract). The kidney is a poor excretory organ, since plasma lead together with other "heavy metals" is largely protein bound.

DISTRIBUTION OF LEAD IN "NORMAL" HUMAN TISSUES—

The distribution of lead[1,18] is shown in Table I. Under "normal" conditions there is a retention of lead. Such retention is so small that it cannot be detected by balance experiment. The high concentrations in bone will be noted. Lynch *et al.*[3] showed that the concentration of lead in such bones as femur and tibia increased with age. I have confirmed

this[18] and also shown that this phenomenon was not exhibited by such bones as rib and vertebra.

BLOOD—

It has been noted that the concentration of lead in blood is much higher than in urine. Accurate analysis can be made with 20 ml or less of blood, quantities that can easily be obtained from a patient. Normal human blood contains on an average 50 μg of lead per 100 ml of whole blood. It has been suggested by Anderson and the author[1] that the determination of blood lead is a more reliable index in plumbism than the determination of lead in urine. Lead exists in red blood cells and plasma, and variations in distribution have been noted,[19] but at present no special significance has been attached to such change.

MOBILISATION OF LEAD—

It is generally agreed that only lead present in the soft tissues exerts a toxic action, that in the skeleton being inert. However, conditions may exist that may produce a transfer of lead from soft tissues to skeleton or *vice versa*. A high calcium diet causes lead to be deposited in the skeleton, whereas a low calcium diet produces the reverse. The hormone of the parathyroid glands[20] and overdosage with vitamin D produce a transfer of lead from the skeleton to the soft tissues.

Acidosis resulting from disease or by the administration of certain salts, *e.g.*, ammonium chloride, also produces a transfer of lead from the skeleton to the soft tissues.

It is believed that lead laid down in the skeleton under so called normal conditions is difficult to mobilise, *i.e.*, a transfer from skeleton to soft tissues. In contrast, large amounts of lead laid down in the skeleton under abnormal conditions may readily be mobilised.

Brown[21] has shown that in persons with no history of abnormal exposure to lead, mobilisation of skeletal lead may occur in diseases associated with decalcification. This was shown by an increase above the normal levels of the blood lead. Reference[22] has also been made to a case of lead poisoning occurring in a lead worker in association with, and probably precipitated by, subacute lymphatic leukaemia.

LABORATORY INVESTIGATIONS IN PLUMBISM—

Haematological examination of blood and measurement of the excretion of coproporphyrins in urine are of considerable value in the investigation of plumbism. However, only laboratory investigations concerned with the determination of lead will be considered. The function of such is to determine whether there has been abnormal ingestion of lead.

TABLE II

EFFECT OF DIET AND TREATMENT ON THE LEVEL OF THE LEAD CONTENT OF BLOOD IN PLUMBISM

Diet and treatment		Lead content of blood, μg per 100 ml
1. No treatment + ordinary diet		350
2. High calcium diet		80
3. Low calcium diet + treatment with ammonium chloride	A	180
	B	1500
	C	3600
4. High calcium diet		70

TABLE III

EFFECT OF DIET AND TREATMENT IN THE EXCRETION OF LEAD IN PLUMBISM

The results represent the average of 3-day periods

Diet and treatment		Lead in urine, mg per day	Lead in faeces, mg per day
1. High calcium diet	A	0·3	0·9
	B	0·2	0·9
2. Low calcium diet + treatment with ammonium chloride	A	0·3	2·0
	B	0·3	1·8
	C	0·2	1·5
3. High calcium diet	A	0·1	1·2
	B	0·3	0·6

TABLE IV

LEAD CONTENT OF TISSUES OF PERSONS WITH A HISTORY OF ABNORMAL EXPOSURE TO LEAD

	Lead content, mg per kg of fresh tissue						
	Liver	Kidney	Brain	Rib	Vertebra	Femur	Tibia
Painter age 41 years ..	4·5	1·0	1·0	119·0	19·0	—	—
Painter age 60 years ..	2·4	2·9	—	22·0	9·0	—	—
Metal worker age 60 years	5·4	2·0	1·4	51·0	13·0	51·0	79·0
Condition due to contamination of drinking water with lead; subject age 26 years..	7·1	4·6	3·1	52·0	—	52·0	53·0

A measure of urinary excretion of lead is often the only practical means of investigation of persons during abnormal exposure, *e.g.*, in factories.

An excretion of more than 100 μg of lead per day in the urine is considered to be suggestive of abnormal exposure to lead. Frequently patients are admitted to hospital when exposure to lead has ceased for a period. In such cases the urinary excretion of lead may be within normal limits and yet there may be mobilisable lead in the skeleton. The determination of the lead content of the blood under various conditions tends to produce the most satisfactory results.[5,23,24] The lead content of the blood may be normal on admission. A low calcium diet together with acidosis, which may be induced by the oral administration of ammonium chloride, may produce an increase in the level of blood lead above the limit of normality (provisional—100 μg of lead per 100 ml of whole blood), in those persons with mobilisable lead in the skeleton. Such levels may then be brought down to within normal limits by a high calcium diet. Such changes should not occur in those persons only subject to the so called normal hazard. Some typical results are shown in Table II. The effect of such treatment upon the excretion of lead is shown in Table III. It will be noted that the main changes occur in faecal excretion.

When available, useful results may be obtained from the analysis of tissues. Some results are shown in Table IV. The markedly increased concentration of lead in rib is noteworthy.

CHELATING AGENTS—

The removal of excess of lead in plumbism has been an important problem. Treatment with a low calcium diet and ammonium chloride or other agents to increase excretion of lead by mobilisation has been considered to be dangerous, since such could potentiate symptoms of plumbism. Alternative procedures have been sought. The use of chelating agents[25] appears to offer considerable promise. The use of calcium disodium ethylenediaminetetra-acetate (EDTA) has been investigated and most promising results have been reported.

I have had the opportunity to examine the lead content of the urine in a man with a history of slight abnormal exposure to lead when subjected to such treatment. Before treatment, the lead content of the urine was 50 to 80 μg per litre, whereas during treatment with EDTA the concentration had an average value of 1·46 mg per litre.

It may be that such substances may not only be of value in the treatment of plumbism but also in diagnosis.

EXPERIMENTS ON ANIMALS—

Although much useful information has been obtained from the study of plumbism in the human, more direct evidence may be obtained from experiments on animals.

Using mice as the experimental subjects,[23,26,27] I studied two aspects, *viz.*, the effect of dietary composition upon the absorption of lead from the alimentary tract; and factors influencing the distribution of lead between the soft tissues and skeleton.

In the first study, the quantity of lead in the whole animal was determined after various regimes. It was found that: (*a*) absorption of lead was least on a high calcium diet and highest on a low calcium diet, and (*b*) the addition of dilute hydrochloric acid to the diet resulted in increased absorption of lead. This could be related to the pH of the intestinal contents, a factor known to influence the absorption of "heavy metals."

In the second study, the distribution of lead between soft tissues and skeleton was examined. It was found that: (*a*) the percentage of the total lead in the skeleton was highest on a high calcium diet, and (*b*) in contrast to controls on a high calcium diet, increased percentages of lead were found in the soft tissues when the animals were maintained on a low calcium diet or treated with sodium bicarbonate or potassium iodide.

METHODS OF ANALYSIS—

Results of analyses of urine, faeces, blood and soft tissues referred to in this paper were obtained by the procedure described in 1935,[1] with the differences in manipulative technique referred to in this paper. Results for the lead content of bone obtained from individuals without a history of abnormal exposure to lead were obtained either by the procedure described in 1935[1] or by the modification described in 1939.[6] No differences were noted between the ranges obtained for the four types of bone examined by the two versions of the method. Bones obtained from individuals with a history of abnormal exposure to lead were determined by the modified procedure described in 1939.[6]

With the exception of urines examined during therapy with EDTA, results referred to in this paper were assessed by visual colorimetry.

DISCUSSION

The dithizone extraction procedure described in this paper was described 20 years ago and, since the subject is of some interest, many related papers have been published.

Although many modifications have been published, the determination of lead with dithizone still appears to be the most popular of the chemical methods. A notable addition has been the introduction of the reversion principle[8] and its use with the photo-electric colorimeter.

Suggestions[10,13,28] for the use of a chloroform solution of diethylammonium diethyldithiocarbamate in place of sodium diethyldithiocarbamate and ether have been made. Such a reagent has advantages, since it may be readily prepared and the solvent is non-inflammable. It also appears to have a greater versatility in the separation of a wide range of trace metals. The recent publication of Gage[11] is of great interest. Sodium diethyldithiocarbamate is used and the lead compound is extracted into *iso*amyl alcohol - toluene. Lead is then specifically extracted from organic solution with aqueous mineral acid, thus saving the evaporation of organic solvent. It is obvious that we still have much to learn with regard to the properties of metallo-organic compounds.

There has been much discussion about the respective merits of the destruction of organic matter by ignition or wet digestion.[11,29]

The spectrograph has not been applied on a large scale, principally because of its cost and because in many laboratories it would only be put to occasional use. The polarograph[30] until recently lacked the sensitivity to deal with the low concentrations present in many biological materials.

There is general agreement with regard to the distribution of lead in human excreta and soft tissues, although there may be local differences. This could be largely related to the lead content of the local water supply or possibly to atmospheric conditions.

When such material is obtainable, a knowledge of the lead content of the various bones comprising the skeleton can give very useful information with regard to abnormal exposure to lead during life. Much more investigation, however, appears to be required on this subject. Although numerous analyses have been published, there appears to be: (*i*) inadequate data to specify the normal range for any particular type of bone and also the effect of local conditions, and (*ii*) inadequate data about the exact relationship between age and the lead content of such bones as femur and tibia. There is also inadequate data about the type of bone that would give the most useful information in the study of plumbism. Since the lead content of bone is relatively high in comparison with other tissues, it would appear that analyses could be carried out by polarography, high sensitivity not being required.

The introduction of chelating compounds may alter the biochemical approach to the diagnosis and control of treatment of plumbism.

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The Photometric Determination of Silicon in Steels

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An improved method, based on the molybdenum-blue reaction, is proposed for the determination of silicon in steels, a mixture of ferrous ammonium sulphate and oxalic acid being used for the reduction. By making measurements at either of two wavelengths, a wide range of silicon contents is covered. A modification to the Spekker absorptiometer permitting it to be used at 805 mμ is described.

FOR the determination of the silicon content of steels, photometric methods have, by virtue of their speed and precision, largely displaced the older gravimetric methods. In this country, the so called "molybdenum-blue" method is probably more widely used than the alternative yellow silicomolybdate method. This is exemplified by the two British Standard absorptiometric methods,[1,2] one covering the "normal silicon range" of 0·05 to 2·0 per cent. and the other the "low silicon range" of 0 to 0·05 per cent. Both of these methods depend upon the determination of the silicon as molybdenum blue, but they differ, among other details, in the choice of reagent for reducing the silicomolybdate.

It is not convenient to have two distinct methods for two ranges of silicon, since this necessitates keeping two different sets of reagents and may lead to confusion and error in routine practice. It would be preferable to have a single method which, by slight modification of the conditions, would permit the precise determination of silicon at all levels. It is usual in absorptiometric analysis to cover a wide range by taking either different sample weights or different aliquots; however, this is not easily possible in molybdenum-blue procedures, since conditions for the colour formation have to be closely controlled. The main objective of the present work was to overcome this difficulty, and this aim has, in fact, been simply achieved by measuring the colour of the molybdenum blue at two wavelengths, one for low silicon contents up to 0·13 per cent. and the other for normal silicon contents up to 3 per cent. The same chemical operations are used in both ranges.

Three reducing agents were considered, but, for reasons to be given, a mixture of ferrous

ammonium sulphate and oxalic acid was eventually chosen. The method finally developed differs in two slight, but nevertheless important, points from that proposed earlier by Gentry and Sherrington.[3] These modifications have been necessitated by the need to determine very low silicon contents. Most of the investigational work has, in fact, been concerned with finding the optimum conditions for determining low silicon contents. These conditions have then been shown to be equally satisfactory for the normal silicon range.

Experimental

There are so many variables in the molybdenum-blue method, as applied in steel analysis, that it is no doubt possible to develop a number of distinct methods—each capable of giving results of good accuracy. For our purposes, a method of good routine-reliability was required, suitable for simultaneous determinations on six or more samples. Although speed of analysis was necessary, even more importance attached to the need to find conditions that were not highly critical, so permitting some reasonable relaxation on the part of the routine analyst.

These considerations eliminated methods requiring heating of the solution to promote the rapid formation of the silicomolybdate, since it has been found that such heating is difficult to control in routine work. Some attention was paid to the possibility of finding conditions under which the silicomolybdate formed rapidly at room temperature. However, it was subsequently realised that this was not necessary or desirable when batches of samples were being analysed.

In one experimental detail we prefer the technique of Vaughan[4] as against that of later workers.[5,6] This concerns the use of a beaker or conical flask in place of a calibrated flask for making up the final colour solution. Experience has suggested that there is little to choose between the two techniques for speed or accuracy, but has served to emphasise the fragility of the 50-ml calibrated flask.

Choice of reducing agent—

Three reducing agents were compared: stannous chloride,[4,5] ferrous ammonium sulphate[3,5] and 1-amino-2-naphthol-4-sulphonic acid.[7] Yet other reducing agents could have been considered, but, since no other reagent has met with wide acceptance in steel analysis, it was obviously helpful to take advantage of the experience gained over a number of years with the reducing agents chosen.

For the stannous chloride method, the British Standard method[1] was used. With ferrous ammonium sulphate, early experiments were carried out by the method of Gentry and Sherrington.[3] For the 1-amino-2-naphthol-4-sulphonic acid method, a procedure previously used in these laboratories was adopted. It was, briefly, as follows—

Weigh 0·5 g of steel into a 250-ml beaker. Add 70 ml of 5 per cent. sulphuric acid and warm until the steel dissolves. Add 100 ml of water and 2·5 g of ammonium persulphate. Boil for 10 minutes. Add 20-volume hydrogen peroxide dropwise until any precipitated manganese dioxide dissolves. Boil for 3 minutes, cool and dilute to 500 ml. Place by pipette two 25-ml portions (A and B) in two dry 250-ml beakers.

Add to A 10 ml of 2·5 per cent. ammonium molybdate solution, mix and set aside for 5 minutes. Add 10 ml of 4 per cent. oxalic acid, and mix. Add 5 ml of the reducing solution and set aside for at least 10 minutes. Add to B 10 ml of 4 per cent. oxalic acid, mix and add 10 ml of 2·5 per cent. ammonium molybdate, mix and then add 5 ml of reducing solution.

Read the optical densities of the solutions on the Spekker absorptiometer, using Ilford No. 608 and Calorex H503 filters with 1-cm cells. Read off differences from a calibration graph.

Prepare the reducing solution by grinding 0·5 g of 1-amino-2-naphthol-4-sulphonic acid to a paste with water. Add 200 ml of water, 35 g of potassium metabisulphite and 6 g of sodium sulphite. Warm gently until solution is complete, dilute to 250 ml and store in a dark bottle.

As applied to plain carbon steels, there was little to choose in the precision attainable with the three methods. In each case the calibration is linear and the sensitivity not very different. Examination of the complete visible absorption spectra of the molybdenum blue produced by the three methods showed no differences.

All three methods have disadvantages. The use of ferrous ammonium sulphate and oxalic acid results in a rather high background colour, which, although corrected for by the

use of a compensating solution, is not desirable when low silicon contents have to be determined. The 1-amino-2-naphthol-4-sulphonic acid reagent shows a tendency to crystallise out of the final solution, as has been mentioned by Mullin and Riley.[8] Although this presents no difficulty if the optical density of the solution is read fairly rapidly, it is an annoyance and possible cause of error in batch analysis. Stannous chloride as a reagent has the minor disadvantage of being rather unstable and thus requiring fresh preparation at least daily. Of more importance is the fact that the stannous chloride methods have been limited to those steels that are soluble in dilute sulphuric acid.

The latter point was the main factor for preferring the ferrous ammonium sulphate - oxalic acid method to the stannous chloride method, Both have been widely favoured by steel analysts, as shown by the co-operative studies of the Glasgow Absorptiometric Panel[6] and the Methods of Analysis Committee of B.I.S.R.A.,[5] but to us the extension in scope consequent on the ability to use a range of acid solvents was an over-riding advantage.

CONDITIONS FOR COLOUR DEVELOPMENT—

Of the several possible variables in the procedure, some have been kept at fixed values based on previous experience.[3] These have included the sample weight and the quantities used of ammonium molybdate, ferrous ammonium sulphate and oxalic acid; all of these factors are easily controlled in individual determinations. The temperature for the formation of the silicomolybdate and its reduction is a critical factor, but throughout the present work it has been kept at 20° ± 3°C. The conditions that were studied in detail were the amount of solvent acid used and the waiting time for the formation of the silicomolybdate complex.

Acidity—The influence of the amount of solvent acid on the method is shown in Fig. 1, which indicates the effect of different amounts of the several solvent acids used in the final method as applied to a steel containing 0·08 per cent. of silicon. Measurements were made on the Unicam spectrophotometer at 810 mμ.

It can be seen from Fig. 1 that, for all three solvents, the maximum optical density

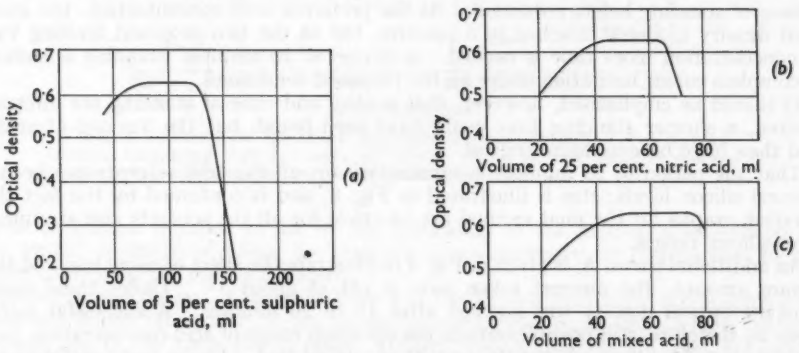

Fig. 1. Effect of concentration of solvent acid: (*a*) 5 per cent. sulphuric acid; (*b*) 25 per cent. nitric acid; (*c*) 20 per cent. hydrochloric acid - 6·5 per cent. nitric acid

is the same within experimental error. This maximum optical density is attainable over quite wide ranges of acid concentration, the permissible variation being 80 to 120 ml of 5 per cent. sulphuric acid, 45 to 65 ml of 25 per cent. nitric acid or 55 to 75 ml of the nitric - hydrochloric acid mixture.

The pH values of the solution were measured with a glass electrode during the stage at which the silicomolybdate was forming. The limiting values of acid concentration for maximum colour formation corresponded to a pH range of 0·72 to 0·92 for the sulphuric acid solution, 0·45 to 0·70 for the nitric acid solution and 0·45 to 0·66 for the mixed-acid solution. Little physical significance probably attaches to pH measurements in solutions as complex as those studied here, but the values are given since they are considerably lower than those generally accepted for the formation of silicomolybdate. Thus Lacroix and Labalade[9] quote a pH range of 1·5 to 1·7, whilst Mullin and Riley[8] say that the rate of formation of silicomolybdate is "much reduced in solutions more acidic than pH 1·0, presumably

owing to polymerisation of the silicic acid." Both sets of workers were, however, dealing with the formation of silicomolybdic acid in more-or-less pure solution, and Mullin and Riley indicate that a higher acidity is necessary in the presence of ferric iron.

Formation of silicomolybdate—The formation of the silicomolybdate is dependent on the time of standing as well as on the acidity. This is illustrated in Fig. 2, which shows, for

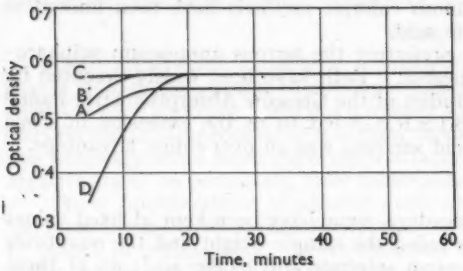

Fig. 2. Effect of time for formation of silicomolybdate: curve A, 30 ml; curve B, 45 ml; curve C, 55 ml; curve D, 65 ml of 25 per cent. nitric acid. All curves are for steels containing 0·08 per cent. of silicon, measurements being made on a Unicam SP500 spectrophotometer in 4-cm cells

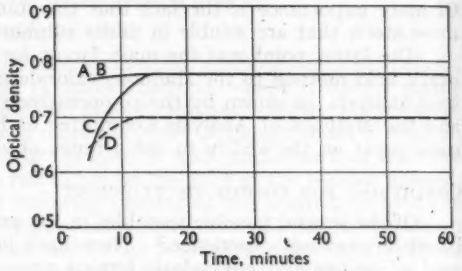

Fig. 3. Effect of time for formation of silicomolybdate as in final method: curve A, sulphuric acid; curve B, nitric acid; curve C, mixed acid; curve D, nitric acid. Curves A, B and C are for steels containing 0·1 per cent. of silicon, measurements being made on a Unicam SP500 spectrophotometer in 4-cm cells; curve D is for a steel containing 2 per cent. of silicon, measurements being made on a Spekker absorptiometer

various amounts of nitric acid, the optical density of the molybdenum blue as a function of the time of standing before reduction. At the preferred acid concentration, the maximum optical density is almost reached in 5 minutes, but at the two proposed limiting values of acid concentration more time is needed. A period of 20 minutes' standing is sufficient to give complete colour formation under all the proposed conditions.

It should be emphasised, however, that acidity and time of standing are inter-related; if desired, a shorter standing time could have been found, but the amount of acid taken would then have become more critical.

That the period of 20 minutes is satisfactory for all the acid solvents has been tested at several silicon levels; this is illustrated in Fig. 3, and is confirmed by the fact that the calibration graphs for the final method are identical for all the solvents and are linear over all the silicon ranges.

An additional curve, A, is given in Fig. 2 to illustrate the effect of using less acid than the optimum amount; the amount taken gave a pH of about 1·0. Under these conditions a constant optical density was reached after 15 to 20 minutes. A successful method of analysis is, therefore, still possible outside the optimum range of acid concentration, provided that rigid control is exercised over the amount of solvent acid. Under such conditions a linear working graph is also obtained.

Stability of the molybdenum-blue complex—Bagshawe and Truman[10] have claimed that the molybdenum-blue complex produced by using a ferrous sulphate - oxalic acid mixture shows a progressive decrease in colour on standing for 1 hour. However, their conditions were markedly different from those used in steel analysis, particularly since they did not control the oxidation potential of their system by the deliberate addition of ferric iron. Under the conditions given in this paper, the coloured complex is very stable and has shown no change when measured at intervals up to 19 hours. This is in agreement with earlier work.[3]

BLANKS—

The determination of small amounts of silicon in steels is complicated by the need to make blank corrections. There are the following three possibilities: (*a*) a reagent blank caused by the presence of small amounts of silicon in the reagents used and derived from the apparatus or atmosphere, (*b*) a colour blank caused by the presence of coloured ions or complexes in the solution, and (*c*) an interfering-element blank. In addition there is the

further complication of obtaining silicon-free iron for use in the preparation of the calibration graph.

For the last purpose, in the British Standard methods[1,2] ferrous sulphate has been used instead of an iron or steel, since it "has been found to give less colour development due to silicon than any form of pure iron commercially available at present." Ferrous sulphate is also used in the British Standard methods to obtain the reagent blank in actual determinations. This is open to two criticisms. Firstly, since ferrous sulphate is used in place of iron, it is necessary to take less solvent acid; thus, a true reagent blank is not obtained. Secondly, it is implicit in the procedure that the ferrous sulphate contains a negligible amount of silicon; if any is present, the result on a steel sample will be too low by the equivalent silicon content. In our experience, these criticisms are not serious when AnalaR reagents are used. Nevertheless, when very low silicon contents are to be determined, it is preferable to use a steel of known low silicon content to obtain the reagent blank. It is also more

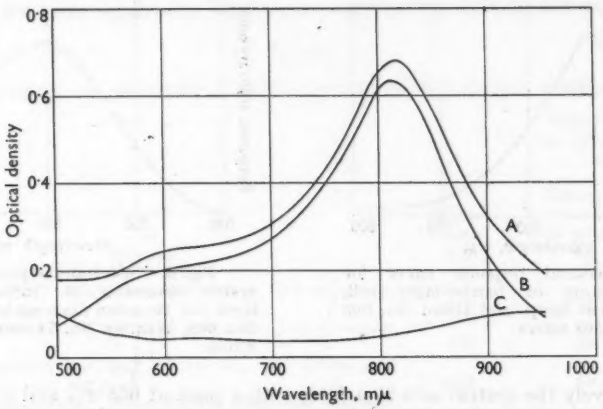

Fig. 4. Absorption spectra of molybdenum-blue complex: curve A, test solution containing 0·06 per cent. of silicon; curve B, test solution minus compensating solution; curve C, compensating solution

convenient in routine practice to use in the calibration of the method a low-silicon steel to which known additions of silicon are made; it is not necessary for this purpose to know the silicon content of the steel accurately.

Contrary to the statement made in Note 6 of the British Standard,[2] the reagent blank, as determined in this laboratory, is not normally exceedingly small and, in fact, correction for it is essential in the lower silicon range. When AnalaR reagents are used, the blank is equivalent to about 0·005 to 0·010 per cent. of silicon. There is some evidence that most of this is due to traces of silicon in the ammonium molybdate, since the lowest blank is obtained when only large clear crystals of ammonium molybdate are selected.

The colour blank due to the presence of coloured ions and complexes is simply corrected for by using a compensating solution, which contains all the reagents but added in an order that does not lead to the formation of silicomolybdate. Since at both wavelengths used for measurement the optical density of the compensating solution for most steels is small and constant, it is not necessary in routine practice to measure it on all samples.

The interfering-element blank has not been studied in this work for the normal silicon range, since no interferences have been reported by previous workers[3,4] under these conditions for measuring the optical density. However, it was necessary to test the low-range method because of the different measuring conditions. For this purpose a series of experiments was conducted on two plain carbon steel samples of different silicon content to which additions were made of the several possible interfering elements in the purest available form. In this manner it was shown that the following were without effect on the method: 0·1% of phosphorus; 0·1% of arsenic; 1% of lead; 5% of copper; 5% of vanadium; 20% of cobalt; 20% of nickel; 20% of chromium; and 20% of manganese.

MEASURING CONDITIONS—

The absorption spectrum from 500 to 1000 mμ of molybdenum blue according to the present method is shown in Fig. 4. It was measured on the Unicam SP500 spectrophotometer, with a 4-cm cell and a slit-width of 0·017 mm. The steel contained 0·06 per cent. of silicon and measurements were made at 10-mμ intervals.

As can be seen, the maximum absorption occurs at a wavelength of 810 mμ, in agreement with the figure found by Mullin and Riley.[3] This value should be compared with the conditions normally adopted when the Spekker absorptiometer is used. Vaughan[4] used the tungsten-filament lamp in conjunction with an Ilford No. 608 and a heat-absorbing filter. By combining the transmission curve of the filters as measured on the Unicam SP500 spectrophotometer with the spectral response of a barrier-layer cell to a tungsten-filament lamp, the spectral response of this system has been obtained. The results, presented in Fig. 5,

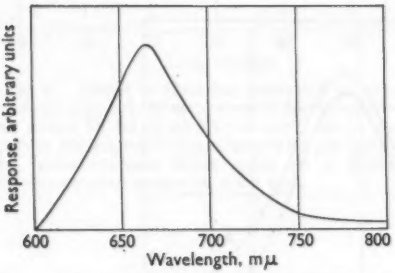

Fig. 5. Spectral response curve for system consisting of barrier-layer cell, tungsten-filament lamp, and Ilford No. 608 and Calorex H503 filters

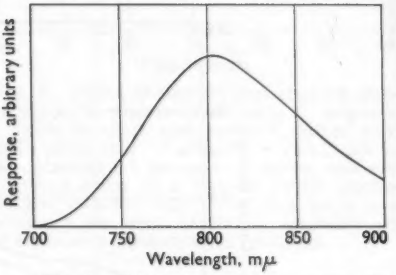

Fig. 6. Spectral response curve for system consisting of "infra-red" barrier-layer cell, tungsten-filament lamp, and Ilford No. 608, Wratten No. 74 and Calorex H503 filters

show that effectively the system acts as a filter with a peak at 665 mμ and a half-band pass of 60 mμ. With the limitations imposed by the barrier-layer cell this represents the best choice of conditions possible on the unmodified Spekker absorptiometer. The Ilford No. 608 and Calorex H503 filters and tungsten-filament lamp combination have therefore been used in the present work for the normal silicon range.

The other commonly used conditions entail use of the mercury-vapour lamp in conjunction with Ilford No. 606 and Calorex H503 filters, which isolate the 577 and 579-mμ lines. Such conditions have been favoured by steel analysts,[5,6] presumably because the mercury-vapour lamp is normally used in the absorptiometric analysis of steels and it is inconvenient to change over to the tungsten-filament lamp for particular determinations.

As can be seen from Fig. 4, the absorption peak of the molybdenum-blue complex occurs at 810 mμ. This is the preferred wavelength for measurements and the one giving the maximum sensitivity. The effect of the background colour is greatly reduced by working at 810 mμ rather than at the more commonly used wavelengths, as can be seen from Fig. 4. In the present work, the low-range method has been based on measurements made at this wavelength with the Unicam SP500 spectrophotometer with a slit-width of 0·017 mm, which corresponds to a nominal band-width of 4 mμ. Some consideration has also been given to modifying the Spekker absorptiometer to permit measurements to be made at this wavelength. For this purpose the barrier-layer cells have been replaced by Megatron barrier-layer cells, "infra-red" type. These cells, in conjunction with the tungsten-filament lamp and filter combination consisting of Wratten No. 74, Ilford No. 608 and Calorex H503, permit measurements to be made at 805 mμ (as can be seen from Fig. 6). Satisfactory calibration graphs have been obtained with the Spekker absorptiometer modified in this way. The commonly used Ilford Spectrum filters show marked transmission in the near infra-red and, if the modified Spekker absorptiometer is to be used for work in the visible region, it will be necessary to verify the calibration of the instrument for the determinations in question. Alternatively, the Calorex H503 filter may be replaced by a suitable filter having a sharp cut-off at about 680 mμ, thereby modifying the over-all response of the photocell - filter

combination to approximate to that of the normal photocell. An interference filter is suitable for this purpose.

Measurements made under any of the conditions discussed give straight-line calibration graphs. By using the Spekker absorptiometer, the tungsten-filament lamp and Ilford No. 608 filters, the range of the method is up to 3 per cent. of silicon with $\frac{1}{2}$-cm cells. On the Unicam SP500 spectrophotometer at 810 mμ, the range is up to 0·13 per cent. of silicon with 4-cm cells or up to 0·5 per cent. with 1-cm cells. The calibration graphs pass through the origin if correction is made for the reagent blank.

The sensitivity of our method, different measuring conditions being used, is shown in Table I, which also gives some results obtained by other methods. It can be seen that by taking measurements at 810 mμ there is a gain in sensitivity of nearly four times over measurements made at 580 mμ. Differences exist between the sensitivity of the several methods when measuring under the same conditions, but this is to be attributed to differences in the conditions for the formation and reduction of the silicomolybdic acid. It is of interest, however, to note that, despite their differences, the present method and the British Standard method[1] have the same sensitivity when measurements are made at 810 mμ.

TABLE I

COMPARISON OF SENSITIVITY WITH DIFFERENT MEASURING CONDITIONS

Method	Wave-length, mμ	Silicon required to give an optical density of 1·00 in a 4-cm cell: Silicon in 50 ml of final solution, μg	Silicon required to give an optical density of 1·00 in a 4-cm cell: Silicon in sample, %
Spekker absorptiometer—			
Present method, Ilford No. 608 filters and tungsten-filament lamp	670	45	0·362
Present method, Ilford No. 606 filters and mercury-vapour lamp	578	62	0·496
British Standard method,[2] Ilford No. 606 filters and mercury-vapour lamp	578	69	0·058
Glasgow Panel method, Ilford No. 606 filters and mercury-vapour lamp	578	58	0·290
Modified Spekker absorptiometer—			
Present method, Ilford No. 608, Wratten No. 74 filters and tungsten-filament lamp	805	20	0·160
Unicam SP500 spectrophotometer—			
Present method	810	16	0·128
Present method	670	43	0·345
Present method	578	60	0·480
British Standard method[1]	810	16	0·080

METHOD

REAGENTS—

Sulphuric acid, 5 per cent.—Carefully pour 50 ml of sulphuric acid, sp. gr. 1·84, into 800 ml of water, mix, cool and dilute to 1 litre.

Nitric acid, 25 per cent.—To 500 ml of water add 250 ml of nitric acid, sp. gr. 1·42, mix and dilute to 1 litre.

Mixed acid—To 500 ml of water add 200 ml of hydrochloric acid, sp. gr. 1·18, and 65 ml of nitric acid, sp. gr. 1·42. Mix and dilute to 1 litre.

Potassium permanganate solution, 2 per cent.—Dissolve 2 g of potassium permanganate in 100 ml of water.

Hydrogen peroxide, 2-volume—Dilute 10 ml of 20-volume hydrogen peroxide to 100 ml.

Ammonium molybdate solution, 2·5 per cent.—Dissolve 2·5 g of ammonium molybdate crystals in 80 ml of warm water, cool and dilute to 100 ml. Store in a polythene bottle.

Oxalic acid solution, 4 per cent.—Dissolve 4 g of crystalline oxalic acid, $(COOH)_2.2H_2O$, in 80 ml of warm water, cool and dilute to 100 ml.

Ferrous ammonium sulphate solution, 6 per cent.—Dissolve 6 g of ferrous ammonium sulphate crystals in 50 ml of warm water. Add 1 ml of 5 per cent. sulphuric acid, cool and dilute to 100 ml.

PROCEDURE FOR SOLUTION OF SAMPLE—

Use the most suitable of the following methods for dissolving the sample—

(*a*) Weigh 0·25 ± 0·05 g of sample (to the nearest 1 mg) into a 350-ml Erlenmeyer flask. Add 100 ml of 5 per cent. sulphuric acid and simmer until solution is complete. Oxidise the hot solution by dropwise addition of 2 per cent. potassium permanganate solution until a permanent precipitate is observed. Add 2-volume hydrogen peroxide dropwise until the precipitate just dissolves. Boil gently for 5 minutes, cool and dilute to 500 ml in a calibrated flask.

(*b*) Weigh 0·25 ± 0·05 g of sample (to the nearest 1 mg) into a 350-ml Erlenmeyer flask. Add 55 ml of 25 per cent. nitric acid and simmer until solution is complete. Add 100 ml of water, boil for 5 minutes, cool and dilute to 500 ml in a calibrated flask.

(*c*) Weigh 0·25 ± 0·05 g of sample (to the nearest 1 mg) into a 350-ml Erlenmeyer flask. Add 65 ml of mixed acid and simmer until solution is complete. Add 100 ml of water, boil for 5 minutes, cool and dilute to 500 ml in a calibrated flask.

PROCEDURE FOR COLOUR DEVELOPMENT—

Place by pipette two 25-ml portions (A and B) in dry 100-ml conical flasks. Add to A from a pipette 10 ml of 2·5 per cent. ammonium molybdate, mix well and set aside for 20 minutes. Add from a pipette 10 ml of 4 per cent. oxalic acid solution, mix well, add immediately from a pipette 5 ml of 6 per cent. ferrous ammonium sulphate solution and mix to give the test solution.

Add from pipettes to B, in this order and mixing between additions, 10 ml of 4 per cent. oxalic acid solution, 10 ml of 2·5 per cent. ammonium molybdate solution and 5 ml of 6 per cent. ferrous ammonium sulphate solution to give the compensating solution.

PROCEDURE FOR TAKING MEASUREMENTS—

Normal range: read the optical densities of the test and compensation solutions on the Spekker absorptiometer, using the tungsten-filament lamp and Ilford No. 608 filters, together with Calorex H503 filters and the most conveniently sized cell. Use water as the reference solution.

Low range: read the optical densities of the test and compensating solution on the Unicam SP500 spectrophotometer in 4-cm cells at 810 mμ with a slit width of 0·017 mm, using water as the reference solution.

Convert the difference between the optical densities of the test solution and the compensating solution to percentage of silicon by reference to the calibration graph. Correct the result for the reagent blank, which is found by treating a steel of very low and known silicon content as described in the procedure.

NOTES ON PROCEDURE—

1. The 5 per cent. sulphuric acid solvent is used for all plain carbon and low-alloy steels. For high-alloy materials the more rapid of the other solvents is used. If the solution after being diluted to 500 ml is cloudy owing to the presence of undissolved carbides, 100 ml should be filtered through a dry filter-paper into a dry beaker.

2. During the colour development and subsequent optical-density measurements, the temperature of the solution should be controlled at 20° ± 3° C.

3. Instruments other than those suggested for measuring the optical density may be used with suitable modifications of the operating details. For the normal range a wavelength of 665 mμ is used and for the low range a wavelength of 810 mμ.

Under the conditions given, the low-range method has a range of up to 0·13 per cent. of silicon with the 4-cm cell. The normal-range method has a range of up to 3 per cent. of silicon with a 0·5-cm cell and up to 0·4 per cent. with a 4-cm cell.

4. The calibration graph is prepared from a low-silicon steel (the silicon content need not be known) to which known additions of a standard silicate solution are made. The preparation of the standard silicate solution is as described in the British Standard.[1] Any of the solvent acids may be used, since they all give the same calibration graph. The series of calibration tests is taken through all the stages of the procedure as described. A blank calibration determination is made on the steel without addition of silicate solution, to allow correction to be made for the silicon content of the steel plus the reagent blank.

RESULTS

A detailed assessment of the normal-range method has not been made, since it differs from the method previously described[3] in only two particulars: the amount of solvent acid and the time allowed for the formation of the silicomolybdate. That these factors have

little effect on the precision has been exemplified by the results obtained in the preparation of the respective calibration graphs. The advantages of the present method over the earlier one are to be found in the less critical conditions rather than in improved precision in carefully conducted experiments.

However, a study of the present low-range method has been necessary, since it is intended for types of steel outside the accurate range of the earlier method. Further, since measurements are made at a widely different wavelength, new or unexpected sources of error were possible.

To test the precision of the low-range method a series of tests has accordingly been carried out by five analysts with three samples. The tests were made under conditions to be expected in routine practice. Hence, although the same calibration graph was used throughout, the several analysts prepared all their own reagent solutions and performed the determinations at different times of day over a period of several weeks. All but one of the operators had had no previous experience of this exact method. The results are given in Table II.

TABLE II

PRECISION OF LOW-RANGE METHOD

Steel	A	B	C
Number of analysts	5	5	5
Number of determinations	30	20	20
Mean result, % of silicon	0·0139	0·0399	0·0439
Range, % of silicon	0·0042	0·0039	0·0066
Standard deviation, % of silicon ..	± 0·0014	± 0·0013	± 0·0015

It can be seen that the probable error is the same at 0·01 per cent. as at 0·04 per cent. of silicon, and over this range there is a 0·95 probability that a single determination is correct to within ± 0·003 per cent. of silicon. This precision is probably limited by the reagent blank, which includes the adventitious pick-up of silicon from glassware and the atmosphere. In the above experiments, the reagent blanks were equivalent to a silicon content in the steel of from 0·005 to 0·010 per cent.

CONCLUSIONS

The method that has been developed fulfils the requirements for a general photometric method of determining silicon in steels. Faster methods can be readily devised for individual types of steel, but the speed of the present method is probably adequate for most purposes. A batch of twelve steels can be analysed in 2½ hours by a single operator. It is not necessary to know the approximate silicon content before a determination is started, since precise results can be obtained over a wide range of silicon contents.

The conditions for carrying out a determination are fairly flexible, provided that care is taken in using pipettes to measure the solutions accurately. It is particularly useful that the volume of solvent acid is not very critical, firstly because this permits measuring cylinders to be used in routine practice and, secondly, because it means that the sample may be dissolved without the risk of error from loss of acid by evaporation. One condition that must be controlled is the temperature of the solution during the formation of the silicomolybdate and the final measurement. It is particularly important that the method should not be used at lower temperatures, since complete formation of the silicomolybdate will not then occur in the time specified.

A limitation to the present method, common to most photometric methods, is that it can be applied in general only to steels that dissolve completely in one of the three solvent acids. Tungsten-bearing and similar steels that give an earth-acid precipitate can be analysed by this method only if it can be established that the precipitate absorbs a negligible amount of silica. When this is so, the photometric method offers very real advantages in speed and precision over any other chemical procedure. However, to establish that no silicon is lost requires a separate investigation of each individual type of steel and this was considered to be outside the scope of the present work. There is need for further work in this connection, as well as in a search for alternative solvents that will give complete solution of such steels.

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The Spectrophotometric Determination of the Alkaline-earth Metals with Murexide, Eriochrome Black T and with *o*-Cresolphthalein Complexone

BY F. H. POLLARD AND J. V. MARTIN

Absorptiometric methods suitable for the determination of microgram quantities of barium, strontium, calcium and magnesium are discussed with reference to the determination of these metals when separated by paper chromatography. Modified procedures are given for the determination of calcium with murexide and of magnesium with Eriochrome black T. New methods for the determination of strontium with murexide and of barium, strontium and calcium with *o*-cresolphthalein complexone are presented.

THE analysis of mixtures of substances by paper chromatography involves the handling of microgram quantities of material, and satisfactory absorptiometric methods were required for the determination of barium, strontium, calcium and magnesium separated by this technique. Absorptiometric methods depending upon preliminary precipitation of a metal as a salt or complex are unnecessary, and may involve considerable losses for such small quantities. Direct methods of determination are preferable.

For barium, strontium and calcium, few direct methods have been reported. Friedrich and Rapoport[1] determined barium as a suspension of the insoluble red rhodizonate in starch or gelatin, but the colour is not very stable and fades rapidly when exposed to light. Ammer and Schmitz[2] reported a yellow coloration for the tannic acid complex of barium (and of strontium and calcium) at a metal concentration as low as 0·1 mg per litre. The colour is unfortunately only transient and fades within a few minutes. There is no direct method for determining strontium, and only one indirect method, of low sensitivity, has been described.[3] Calcium has been determined in drinking water and in blood serum with 2:3:4-trihydroxybenzoic acid (pyrogallol-4-carboxylic acid).[4,5,6,7,8] The reagent in solution is rapidly oxidised by air, and a protective colloid (starch or gum arabic) is required to solubilise the bluish-purple calcium salt. The determination is not very sensitive, the lower limit being about 0·05 mg of calcium per ml.

Several lake-forming reagents of high sensitivity have been used for the direct determination of magnesium, including *p*-nitrobenzeneazoresorcinol (Magneson I),[9] *p*-nitrobenzeneazo-1-naphthol (Magneson II),[10] 3-hydroxy-1-*p*-nitrophenyl-3-phenyltriazine[11] and the Thiazole yellow dyes. The reagents of widest application are the Thiazole yellow dyes.[12,13] It is difficult to obtain reproducible results with lake-forming reagents because of factors

such as variation between different dyestuff samples, impurities in the protective colloids, rapid fading of the magnesium complex and the uncertain effect of other metals.

The most satisfactory reagents for absorptiometric analysis are those that give a true solution of the metal complex. The accuracy and reproducibility of optical-density readings are much less when the complex is insoluble and is held in solution by a protective colloid. The only organic reagents forming true solutions with the alkaline-earth metals are certain metal indicators that have recently been widely used in complexometric titrations, *i.e.*, murexide, Eriochrome black T and *o*-cresolphthalein complexone.

Murexide (*ammonium purpurate*)—Murexide has been used by Ostertag and Rinck[14] and Raaflaub[15] for the absorptiometric determination of calcium in nearly neutral solution. The yellow-orange and red-orange forms of the calcium complex are present, but absorb less strongly than the red form, which is predominant at pH values greater than 9 or 10. Hence the sensitivity of the absorptiometric determination is higher in more alkaline solution. Unfortunately the murexide itself becomes less stable under these conditions, but Tammelin and Mogensen[16] have obtained satisfactory results at a pH of 11 maintained by the use of piperidine. Williams and Moser[17] worked at a pH of 11·3 and used the appropriate amount of sodium hydroxide, but special precautions are necessary to compensate for the rapid decomposition of the reagent.

Eriochrome black T [1-(1-*hydroxy*-2-*naphthylazo*)-2-*hydroxy*-5-*nitro*-4-*naphthalenesulphonic acid*]—Dirscherl and Breur[18] and Harvey, Komarmy and Wyatt[19] have reported absorptiometric methods for determining magnesium. Calcium must first be removed by precipitation, since it interferes. The determination is made at pH 10 in a solution buffered with ammonium hydroxide - ammonium chloride. By working at a pH between 7 and 8, the interference of calcium can be eliminated, but the molar absorbancy of magnesium itself is much lower. Young, Sweet and Baker[20] have determined calcium and magnesium simultaneously by using two different pH values (9·5 and 11·7) and a fixed wavelength (630 mμ).

o-*Cresolphthalein complexone* [2:6-*xylenolphthalein*-α:α′-*bis*(*imino*)*diacetic acid*]—*o*-Cresolphthalein complexone or metalphthalein[21] has but recently been synthesised and used for titrations.[22] No absorptiometric procedures have yet been reported for the reagent, although it has very recently been used for the spectrophotometric titration of calcium and magnesium (as also have murexide and Eriochrome black T[23,24]). With other metal indicators, strontium and barium must be determined by replacement titration, since the metal complexes are unstable and not highly coloured.[25,26] A direct titration procedure can be used for these metals with *o*-cresolphthalein complexone. The complexes of strontium and barium are therefore very high coloured and suitable for an absorptiometric determination.

The extinction curves for the alkaline-earth complexes and the unsubstituted reagent are very similar in respect of the position of the bands and differ only in the height of the bands.

Method

Reagents—

Standard barium solution—Analytical-reagent grade barium chloride, $BaCl_2.2H_2O$, in water, containing 100 mg of barium per litre.

Standard strontium solution—Analytical-reagent grade strontium chloride, $SrCl_2.6H_2O$, in water, containing 100 mg of strontium per litre.

Standard calcium solution—Analytical-reagent grade calcium carbonate in approximately 0·01 *N* hydrochloric acid, containing 100 mg of calcium per litre.

Standard magnesium solution—Analytical-reagent grade magnesium chloride, $MgCl_2.6H_2O$, in water, containing 100 mg of magnesium per litre. This solution is standardised by the gravimetric pyrophosphate procedure.

Sodium hydroxide, 0·1 N, *standardised*.

Ammonia solution, dilute—A mixture of 5 per cent. v/v of ammonia solution, sp.gr. 0·880, and 95 per cent. v/v of water.

Buffer solution A—A 0·75 per cent. w/v solution of analytical-reagent grade ammonium chloride in dilute ammonia solution.

Buffer solution B—A 0·24 per cent. w/v solution of analytical-reagent grade ammonium chloride in dilute ammonia solution.

Murexide indicator solution—Murexide is dissolved in 30 parts by volume of water,

the solution is filtered through a coarse sintered-glass funnel and 70 parts by volume of ethanol are added.

Eriochrome black T indicator solution—The solid dye is dissolved in methanol by warming, and the solution is filtered.

o-*Cresolphthalein complexone indicator solution I*—A 0·03 per cent. w/v solution of *o*-cresolphthalein complexone (called "Phthalein Complexone" by Anderegg, Flashka, Sallmann and Schwarzenbach[22]) in a mixture of 28 per cent. v/v of buffer solution A and 72 per cent. v/v of water.

o-*Cresolphthalein complexone indicator solution II*—A 0·1 per cent. w/v solution of *o*-cresolphthalein complexone in a mixture of 28 per cent. v/v of buffer solution B and 72 per cent. v/v of water.

o-*Cresolphthalein complexone indicator solution III*—A 0·1 per cent. w/v solution of *o*-cresolphthalein complexone in a mixture of 28 per cent. v/v of dilute ammonia solution and 72 per cent. v/v of water.

All indicator solutions are freshly prepared each day. Water is redistilled in a steamed-out hard-glass apparatus having ground-glass joints. Standard alkaline-earth, stock buffer and other solutions are stored in steamed-out Jena-glass bottles.

APPARATUS—

A Unicam SP500 spectrophotometer with 1-cm glass cells is used for all optical-density measurements.

ABSORPTIOMETRIC PROCEDURES WITH MUREXIDE—

The method of Williams and Moser for calcium is modified to give more reproducible results, and the linearity range of the calibration graph is increased. A similar method is used for the determination of strontium.

Determination of calcium—The determination is performed at pH 11·3 and at a wavelength of 506 mμ. The method of preparation of the solutions is modified and carefully standardised, since that recommended by Williams and Moser was found to give random optical-density readings.

The solution containing calcium is diluted to about 50 ml in a 100-ml calibrated flask, and a calculated volume of 0·1 *N* sodium hydroxide solution is added to give a final normality of alkali of 0·002 *N*, *i.e.*, pH 11·3. The mixture is diluted to a point just short of a 90-ml graduation mark and shaken. Then a 10-ml volume of 0·016 per cent. w/v murexide indicator solution is added carefully so that it floats on the surface of the lower aqueous layer and does not mix; a 10-ml volume of 0·016 per cent. w/v murexide indicator solution is immediately added to a blank solution similarly prepared. A few drops of water are carefully added to each flask to make up to the 100-ml mark. The two solutions are mixed simultaneously and shaken manually for 5 minutes. Optical-density readings are taken immediately.

Notes on procedure—

1. The blank and the solutions containing calcium are prepared simultaneously, and differential errors due to the high rate of decomposition of murexide are thus eliminated.

2. After the alcoholic murexide and aqueous layers have been mixed, no further dilution with water is needed, and equilibria existing between the various forms of murexide and its calcium complex are established in the volume of solution for which the determination is made. This is important if the equilibria are not rapidly adjustable, since if once established at a lower volume they may not immediately be shifted on dilution of the solution.

Differential errors in murexide decomposition may also occur if the volumes of mixing are not standardised.

3. Williams and Moser found that murexide is most stable in 70 per cent. ethanolic solution. The ethanolic murexide layer is diluted with only a few drops of water before mixing the layers, and the effect on its stability is therefore only slight.

The calibration graph is linear between 0 and 1·2 mg of calcium per litre and curves towards the concentration axis for higher concentrations of calcium. The slope of the linear portion is 0·309 optical-density units per mg of calcium per litre, and is a direct measure of the sensitivity of the method.

The linearity range of the calibration graph may be increased if the concentration of murexide is increased. When 25 ml of 0·02 per cent. w/v murexide indicator solution are used, the graph is linear between 0 and 3·5 mg of calcium per litre and is of slope 0·325 optical-density units per mg of calcium per litre. With a larger volume of ethanol present, a slight volume contraction occurs when the two liquid layers are mixed. The solutions are therefore made up to the mark with water after the layers have been mixed.

Determination of strontium—Strontium is determined at pH 11·3 and a wavelength of 515 mμ by a procedure similar to that used for calcium. For this determination 25 ml of 0·025 per cent. w/v murexide solution are used and give an optical-density graph that is linear between 0 and 10 mg of strontium per litre. The slope of the graph is 0·0792 optical-density units per mg of strontium per litre.

The optical density for barium is less than one-fifth of that for the corresponding concentration of strontium, and the method is too insensitive for the quantitative determination of the metal on a paper chromatogram.

ABSORPTIOMETRIC PROCEDURE WITH ERIOCHROME BLACK T—

Determination of magnesium—The procedure of Harvey, Komarmy and Wyatt[19] for the determination of magnesium is modified. The technique for preparing solutions is standardised, and the amount of Eriochrome black T is increased to give a calibration graph that is linear over a suitable range of optical density.

Harvey *et al.* claimed to obtain a linear optical-density graph between 0 and 1·4 mg of magnesium per litre with 4 ml of 0·1 per cent. w/v Eriochrome black T solution in methanol. This is even less than the theoretical amount needed to form a complex with the magnesium at the higher concentration. A curve bending towards the concentration axis is obtained under these conditions. For this determination 10 ml of 0·1 per cent. w/v Eriochrome black T solution are required to give a graph that is linear between 0 and 1 mg of magnesium per litre. On certain occasions slightly erroneous readings were obtained. Equilibrium in solution was not established immediately in these cases. A mixing technique similar to that used for calcium is employed to eliminate such errors.

The required volume of magnesium solution is put by pipette into a 100-ml calibrated flask, 25 ml of buffer solution A are added and the solution is diluted to a point just short of a 90-ml graduation mark and shaken. Then 10 ml of 0·1 per cent. Eriochrome black T solution are added carefully. The solution is shaken to mix, and made up to the 100-ml mark with water. The optical density is measured immediately at 520 mμ against that of a blank solution similarly prepared, but containing no magnesium.

For purposes of convenience the same buffer solution is used as that used in calcium - *o*-cresolphthalein complexone determinations. The pH value for the determination is 10·15. The slope of the linear portion of the calibration graph is 0·888 optical-density units per mg of magnesium per litre.

ABSORPTIOMETRIC PROCEDURES WITH *o*-CRESOLPHTHALEIN COMPLEXONE—

o-Cresolphthalein complexone was initially investigated as a reagent for the absorptiometric determination of barium, for which there was no direct method of sufficiently high sensitivity. It was, however, found that not only is *o*-cresolphthalein complexone very satisfactory for the determination of barium, but that it is also much more sensitive than murexide for strontium and calcium.

Determination of calcium—The method of preparation of the solutions is again extremely important if reproducible calibration graphs are to be obtained. Methanol and ethanol were examined as solvents for the reagent, but optical-density values were low and were not reproducible. The reagent must be dissolved in an ammonia - ammonium chloride buffered solution. Optical-density readings are taken at pH 10·15 and a wavelength of 575 mμ.

The required volume of calcium solution is put by pipette in a 100-ml calibrated flask; 25 ml of buffer solution A are added and the solution is diluted to a point just short of a 90-ml graduation mark and shaken to mix. Then 10 ml of *o*-cresolphthalein complexone indicator solution I are added, the solution is shaken, made up to the 100-ml mark with water, and shaken again. The optical density is measured immediately against a similarly prepared blank solution containing no calcium.

Notes on procedure—

1. The pH of the *o*-cresolphthalein complexone solution is identical with that of the calcium-containing buffer solution diluted to 90 ml. When the two solutions are mixed, further dilution with only a few drops of water is needed. The importance of the pH and dilution factors may again be explained in terms of delayed equilibria.

2. Optical-density readings fall gradually with time, owing to decomposition of the metal complex. Optical density values must be measured within 15 minutes of preparing the solutions. The solutions are therefore prepared in batches of three with a freshly prepared blank solution for each batch.

3. Irregular optical-density values are obtained when the solutions are vigorously shaken. The amount of shaking employed must therefore be no more than is needed to render the solution homogeneous.

The calibration graph is non-linear and curves away from the concentration axis. The curvature is only slight, and the optical-density value of 0·711 for 1 mg of calcium per litre gives an indication of the sensitivity.

Determination of strontium—Anderegg *et al.*[22] stated that the strongly coloured strontium and barium complexes are formed only at higher pH values, the optical density for barium at pH 10 being only one-third of the optical density at pH 11. To obtain reproducible optical-density graphs for strontium and barium, the pH and reagent concentration must be higher than for calcium.

The strontium determination is made at pH 10·6 and a wavelength of 575 mμ. The method of preparation of solutions is as for calcium, but the buffer solution B and *o*-cresolphthalein complexone solution II are used.

The calibration graph is linear between 0 and 2·8 mg of strontium per litre and of slope 0·373 optical-density units per mg of strontium per litre.

Determination of barium—Optical-density measurements are made at pH 11·3 and a wavelength of 575 mμ, the procedure being as for strontium and calcium. Dilute ammonia solution and *o*-cresolphthalein complexone indicator solution III are used. The barium solution must be diluted to 50 ml before addition of the ammonia solution. The determination must be made as quickly as possible after the addition of the ammonia solution. Excessive shaking must be avoided. These precautions may be necessary because of a tendency for barium to be precipitated as carbonate in solutions of such high alkalinity.

The optical-density graph is linear between 0 and 5 mg of barium per litre, the slope being 0·239 optical-density units per mg of barium per litre.

Conclusions

The absorptiometric procedures described in this paper are of very high sensitivity: 0·5 μg of magnesium, 0·75 μg of calcium, 1·5 μg of strontium and 2·0 μg of barium may be determined to within 10 per cent. They are independent of chromatographic analysis in that they may be used for the determination of alkaline earths separated by other means.

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The Quantitative Analysis of the Alkaline-earth Metals by Paper Chromatography

BY F. H. POLLARD, J. F. W. MCOMIE AND J. V. MARTIN

A paper-chromatographic procedure is described for the quantitative analysis of microgram amounts of mixtures of barium, strontium, calcium and magnesium. The metals are separated as formates by downward elution on a twin-strip chromatogram of acid-washed Whatman No. 1 filter-paper, with a solvent consisting of methanol, *iso*propanol, formic acid, water and ammonium formate. They are extracted and determined spectrophotometrically —barium, strontium and calcium with *o*-cresolphthalein complexone and magnesium with Eriochrome black T.

The method is particularly applicable to the detection of small amounts of magnesium, which may be determined in as much as a 500-fold excess of calcium with an error of less than ± 2 per cent.

Dolomite and limestone mineral samples have been successfully analysed.

ALTHOUGH several paper-chromatographic procedures[1,2,3,4,5,6,7,8,9,10,11] have been reported for the separation of the alkaline earths, little attention has been paid to the accurate determination of these metals. Miller and Magee[3] have determined strontium by visual comparison, and Seiler, Sorkin and Erlenmeyer[6] have used the area-measurement method for the determination of calcium and magnesium in natural waters.[6] These methods are very rapid, but they are essentially only semi-quantitative. Tristram and Phillips[11] have separated calcium and magnesium chlorides, and determined the metals indirectly by micro-titrating the equivalent of silver (from silver chloride) against ammonium thiocyanate; 100-μg to 10-mg amounts of the metals were determined with an error of 5 to 10 per cent. The titration method is not sufficiently sensitive for the determination of smaller amounts of the metals. The analysis of unfavourable ratios of the metals was not studied.

In the preceding paper (p. 348), absorptiometric procedures have been described by which 5 to 10 μg of each alkaline-earth metal may be determined to within ± 2 per cent. Barium, strontium and calcium are determined with *o*-cresolphthalein complexone and magnesium with Eriochrome black T. These reagents are used in this paper in conjunction with a paper-chromatographic separation for the determination of microgram quantities of barium, strontium, calcium and magnesium in synthetic mixtures and in minerals. None of the eluting solvents previously reported gives a quantitative separation of all four alkaline earths and a new solvent was therefore sought.

EXPERIMENTAL

APPARATUS AND GENERAL TECHNIQUE—

The downward-elution method is used, the apparatus for which has been described by Elbeih, McOmie and Pollard.[12]

A pencil line is drawn 8 cm from one end of a 40 to 50-cm sheet or strip of Whatman No. 1 filter-paper. Spots (0·5 cm in diameter) of the solutions to be chromatographed are introduced from fine capillary tubes at 2-cm intervals along the line. After being dried in the air for 15 to 20 minutes, the paper is inserted into the eluting solvent. The chromatographic vessel must be pre-saturated with the solvent for not less than 6 hours. When

the solvent has percolated about 30 cm down the paper, the chromatogram is removed and dried by pegging to a glass bar in an electrically heated cupboard.

On untreated paper, barium and strontium are detected by spraying with sodium rhodizonate. Calcium is detected with pyrogallol-4-carboxylic acid and magnesium (and beryllium) with 8-hydroxyquinoline. On acid-washed paper, 8-hydroxyquinoline is very sensitive for all the metals that emit a green fluorescence when held over ammonia under ultra-violet light. The fluorescence of the spots on untreated paper is masked by the strong background fluorescence due to calcium and magnesium impurities. In the absence of ammonia, the fluorescence of the barium spot fades rapidly, and so use of sodium rhodizonate is preferable, since the colour produced is permanent.

The detecting reagents used are: a 0·5 per cent. w/v solution of 8-hydroxyquinoline in a 60 per cent. v/v industrial methylated spirit - 40 per cent. water mixture; and a 0·1 per cent. w/v aqueous solution of sodium rhodizonate.

THE ELUTING SOLVENT—

A solvent system for the separation of the metals on Whatman No. 1 filter-paper was found by starting from a simple three-component mixture of acetone, acetic acid and water. The metal salts corresponding to the acid or anionic species in the solvent were used to prevent the metals from forming more than one spot.[13] The addition of ammonium acetate to the solvent reduced the tailing of the barium and strontium. The replacement of acetic acid and ammonium acetate by formic acid and ammonium formate, respectively, gave further improvement in the separations. A number of water-miscible organic liquids was examined, including acetone, dioxan, *iso*propanol, ethanol, methanol and *tert.*-butanol, and the solvent finally selected consisted of 50 ml of methanol, 30 ml of *iso*propanol, 2 ml of formic acid, 15 ml of water and 2·5 g of ammonium formate.[14] The R_F values of the alkaline earths in this solvent are: barium, 0·30; strontium, 0·45; calcium, 0·60; magnesium, 0·75 (see Fig. 1). The solvent flows 30 cm past the starting line in about 6 hours. The chromatographic vessel must be kept in a cool place where the temperature does not rise above 18° C.

A quantitative separation of magnesium and beryllium is not obtained with the above solvent, but may be readily effected with many other solvents of the same five-component system, *e.g.*, 35 ml of methanol, 45 ml of *iso*propanol, 2 ml of formic acid, 15 ml of water and 2·5 g of ammonium formate.

The presence of an ammonium salt in the solvent results in the formation of a second front or boundary on the chromatogram. This front is detectable when the moist chromatogram is sprayed with pH indicators, which show that the leading zone is acidic and the rear zone is nearly neutral. Burstall, Davies, Linstead and Wells[1] have suggested that such a front is produced by the abstraction of water from the solvent by the hydrophilic cellulose, and that the leading zone is essentially free from water. A phase separation or "salting out" occurs, and is caused by the presence of a highly polar species that is not soluble in the water-impoverished organic solvent. Ammonium formate moves in the water-rich phase and formic acid in the water-impoverished phase.

Barium and strontium move in the rear zone, and the elimination of tailing appears to be connected with the buffering action of the ammonium salt.

PURIFICATION OF THE FILTER-PAPER—

Filter-paper contains considerable quantities of impurities, such as silica, alumina, ferric oxide, calcium oxide and magnesium oxide, and traces of other metals, such as lead and copper. The bulk of these impurities may be extracted by soaking the paper in a dilute mineral acid, but the last traces are exceedingly difficult to remove.[15,16,17,18,19] Untreated Whatman No. 1 filter-paper contains approximately 1 μg of calcium per sq. cm, and subsequent washing with dilute hydrochloric acid reduces the calcium content to 0·1 μg of calcium per sq. cm. Special quantitative Whatman filter-papers, Nos. 40, 540, 541 and 542, contain a slightly greater amount of calcium. Many methods were studied for the purification of filter-paper, but the interference of calcium impurities in quantitative determinations could not be entirely eliminated. It appears that the chromatographic solvent continuously dissolves away small amounts of the cellulose itself during an elution and thus sets free metallic impurities not previously removed in the washing procedure. The calcium and magnesium impurities thus solubilised are not uniformly distributed over the chromatogram, but are concentrated in a zone just ahead of the second front.

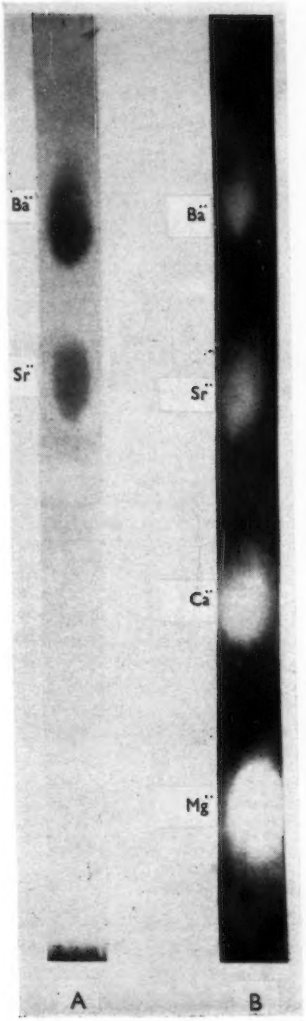

Fig. 1. Separation of the alkaline earths: detecting reagent for strip A, sodium rhodizonate; detecting reagent for strip B, 8-hydroxyquinoline (viewed under ultra-violet light)

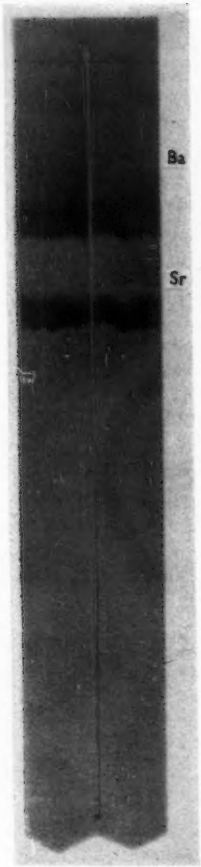

Fig. 2. Twin-strip quantitative chromatogram: detecting reagent, sodium rhodizonate

[To face p. 354

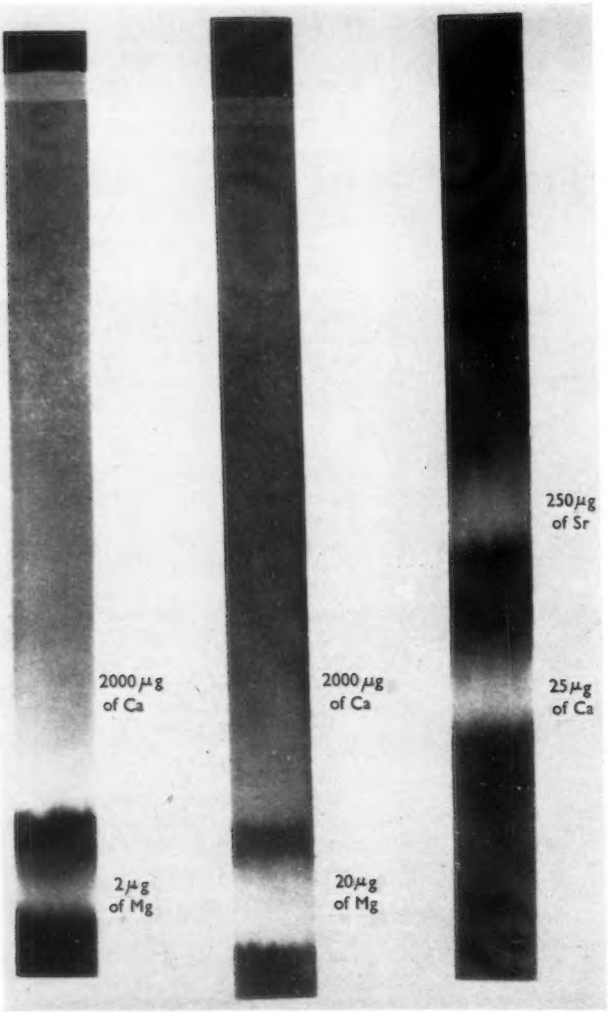

Fig. 3. Quantitative analysis of binary alkaline-earth mixtures: detecting reagent, 8-hydroxyquinoline (viewed under ultra-violet light)

The following method of purification is used. Four sheets of Whatman No. 1 filter-paper, 23 cm × 56 cm, are soaked for not less than 5 days in about 1·5 litres of a (1 + 5) mixture of analytical-reagent grade concentrated hydrochloric acid and water contained in a rectangular porcelain dish (34 cm × 29 cm × 7 cm). The acid is removed by decanting, and the papers are washed with successive batches of water until the supernatant liquid fails to give a test for chloride ions with silver nitrate. The sheets are carefully removed and dried by pegging to a glass bar in an electrically heated cupboard. The end of the sheet in contact with the pegs is cut away lest it should have acquired any impurities.

PURITY OF WATER AND THE COMPONENTS OF THE ELUTING SOLVENT—

The water used for all work, including the acid-washing of the filter-paper, was redistilled in a steamed-out hard-glass apparatus having ground-glass joints. The calcium content of the water was less than 0·01 μg per ml.

The calcium content of the components of the solvent was negligible. Analytical-reagent grade formic acid contains about 2 μg of calcium per ml, but the error involved may be neglected, since it is present in only 2 per cent. amount by volume in the eluting solvent.

THE QUANTITATIVE CHROMATOGRAM—

The chromatogram (see Fig. 2) consists of twin strips, each 4 cm wide and 45 cm long, divided by a 0·2-cm slot. From a calibrated Agla micrometer syringe identical volumes of solution are delivered along the two sections of the starting line. The wet bands must be no wider than 1·2 cm, corresponding to 0·025 ml of solution. For larger volumes, the solution is put on to the chromatogram in successive 0·02 or 0·025-ml aliquots, the chromatogram being dried at about 40° C for 2 to 3 minutes between each addition. The chromatogram is dried, inserted into the solvent and eluted overnight for 14 to 16 hours. The solvent front in this time flows past the end of the chromatogram. The greater distance of flow gives an increase in the distance of separations of the metal zones. After being dried, the chromatogram is cut into its two component strips, one of which—the pilot strip—is sprayed with detecting reagent. The position of the metal zones is marked on the other strip—the sample strip—and the bands are excised by cutting along the horizontal broken lines.

By using connected strips of exactly the same dimensions, equal solvent flow rate and rate of movement of the metals on the sample and pilot chromatograms is ensured. The end of each strip is cut to a point to give an even flow of solvent from the chromatogram. When flat ends are used, the solvent tends to drip from the outer corner of each strip, and sometimes magnesium "creeps" down the outer edge.

To concentrate a small quantity of one metal in the presence of a large amount of another metal that moves to a lower R_F value, a tapered twin-strip chromatogram may be used. The strips are tapered from a width of 4 cm to a width of 2 cm at a distance of 20 cm from the starting line. The width is constant (2 cm) for the lower 25 cm of the strips. The metal is concentrated into a smaller area of paper, for which the blank correction is correspondingly less. This type of chromatogram is used for the determination of small amounts of magnesium in the presence of calcium. The strips should not be tapered to a greater extent, otherwise the solvent flows too rapidly and the metal bands overlap.

EXTRACTION OF THE METALS FROM THE CHROMATOGRAM—

Strontium, calcium and magnesium—Calcium and magnesium may be extracted quantitatively from the filter-paper by two methods—

(*i*) The filter-paper containing the alkaline earth is cut into small pieces, placed in a 50-ml Pyrex-glass conical flask and covered with about 15 ml of 1 per cent. formic acid. After 2 hours, the supernatant liquid is poured into a 100-ml Pyrex-glass conical flask, and the paper is washed for 10-minute periods with three successive 15-ml portions of water. The total extract is evaporated to small bulk over a low flame, transferred to a platinum crucible and evaporated to dryness under an infra-red lamp. The residue contains alkaline-earth metal, soluble organic matter and small particles of cellulose. The crucible is heated very gently over a low bunsen-burner flame. The residue chars and, when the carbon has just burned away, the flame is immediately removed. On cooling, an appropriate volume of 0·01 *N* hydrochloric acid is added to dissolve the alkaline-earth metal, and after 15 minutes the liquid is transferred to a calibrated flask

for absorptiometric determination. The concentration of hydrochloric acid is low enough not to produce a sensible change in the pH of the buffered solution for absorptiometric determination. If the volume of the solutions is to be 100 ml, then 10 ml of acid are used. If the volume is to be 10 ml, then 1 ml of acid is used.

Organic matter interferes with absorptiometric analysis and must be removed by burning, since heating to fumes with perchloric acid is not satisfactory. The temperature at which the organic material is removed is critical. If heated too strongly, the formates of the alkaline earths decompose and the metals may not then be quantitatively redissolved in the dilute hydrochloric acid.

(*ii*) The filter-paper band is folded and placed in an open platinum crucible, which is heated just sufficiently strongly to burn away the paper. The residue is dissolved in 0·01 *N* hydrochloric acid as before.

This procedure is much more rapid than (*i*). The heating is again carefully controlled. The crucible is heated to a somewhat higher temperature, but the contact of the material with the surface of the crucible is less intimate than in (*i*), where it is present as a thin evaporated film.

Strontium can only be quantitatively recovered by method (*ii*).

Barium—Barium can not be quantitatively recovered by methods (*i*) or (*ii*). Crystals of pure barium formate melt with charring when heated gently, and at higher temperatures the carbon burns away, leaving a fine white residue insoluble in water. The low recovery values for barium are due to this decomposition, and the residue formed, even after being heated to fumes with strong acids, such as concentrated hydrochloric acid, aqua regia and perchloric acid, is not soluble in water.

The recovery value is still low when the barium formate is heated to fumes with concentrated hydrochloric acid (to convert to the water-soluble chloride) before being heated to destroy organic material. This may only be explained by assuming the barium is combined in some manner with the soluble organic matter extracted from the paper.

Barium is quantitatively recovered only when the heating procedure is omitted. Hence the organic impurities cannot be destroyed, and consequently the absorptiometric blank reading is rather high (5 to 10 per cent. of the barium reading for 200 μg of barium). The following method is used—

Barium formate is extracted from the chromatogram with 1 per cent. formic acid as in method (*i*) (see p. 355). The extract is filtered through a medium-grain sintered-glass funnel to remove solid particles, and then evaporated to small bulk (not to dryness) over a low bunsen-burner flame. The liquid is transferred to a platinum crucible, evaporated to dryness under an infra-red lamp, and the residue is taken up in water. After 15 minutes, the solution containing barium is transferred to a calibrated flask for absorptiometric determination.

The barium on the pilot strip is detected with sodium rhodizonate. To ensure quantitative recovery, the barium zone must be excised 3 cm behind the apparent rear of the spot.

BLANK CHROMATOGRAMS—

The chromatographic solvent removes small quantities of calcium from the filter-paper, and it is necessary to apply a blank correction to values obtained for alkaline-earth samples.

A three-limbed chromatogram, similar to the twin-strip chromatogram, was first used, one limb being the pilot, one the sample and one the blank chromatogram. The flow rate on each limb was not, however, always the same. The blank strip is therefore eluted separately, but under the same conditions. It is cut from the same sheet of filter-paper as the twin-strip chromatogram and from an adjacent position on the sheet. This corrects, as far as is possible, for variation in the impurity content of different samples of the same grade of filter-paper. After elution, a zone of filter-paper is excised from the blank chromatogram and extracted. The area and position of the zone correspond to those of the metal zone on the sample chromatogram.

For a blank correction to be accurate, the solubilisation and movement of the impurities in the paper must be independent of the presence of the alkaline-earth metals introduced on to the chromatogram. For magnesium and calcium determinations, the blank corrections are not entirely satisfactory. When the amount of magnesium on the chromatogram is 20 μg or more, the recovery is 100 per cent. without a blank correction being made. In fact,

when such a correction is applied this value may be only 90 per cent. or less. Magnesium moves to the same position on a chromatogram as would the zone of extracted alkaline-earth impurities. It is therefore probable that the high local concentration of magnesium in the solvent depresses the tendency of the impurities to dissolve. When smaller amounts of magnesium are used, some impurity does dissolve, since the recovery values are greater than 100 per cent. A correction cannot be made for such amounts, since the impurity dissolved on the magnesium-containing chromatogram is less than on the blank chromatogram. The minimum amount of magnesium that can be accurately determined is therefore 20 μg, or 10 μg for a tapered chromatogram.

For calcium determinations, more impurity moves with the calcium on the sample chromatogram than moves in the corresponding position on the blank chromatogram, and recovery values are still too high after applying a blank correction (+ 2 to 4 per cent. for 100 μg of calcium). The application of a further empirical correction factor, such as was used by Ebel,[20] appears to be necessary.

Analysis of synthetic mixtures—

Synthetic binary alkaline-earth mixtures were prepared from solutions of the metal formates standardised absorptiometrically. The separation of small amounts of magnesium from large amounts of calcium and of small amounts of calcium from large amounts of strontium was studied (see Fig. 3). The separation of magnesium is independent of the amount of calcium, and the metal can be determined to within $\pm$ 2 per cent. for amounts greater than 20 μg (10 μg for a tapered chromatogram). Magnesium can be determined with this accuracy in up to a 500-fold excess of calcium. More unfavourable ratios could probably be analysed, the only limiting factor being the time taken in placing on the chromatogram the larger volumes of liquid required.

The separation of small amounts of calcium from strontium is not independent of the amount of strontium. For amounts of strontium greater than 500 μg, the chromatogram is so heavily loaded that some of the metal does not move from the starting line. In such cases the calcium zone is ill defined and recovery values are much less than quantitative, part of the metal being held back by the strontium. For amounts of calcium less than 25 μg, the blank correction is about 10 per cent., so that even an empirical correction factor will not give a sufficiently accurate result. The limiting excess of strontium that may be tolerated, if an accurate value for calcium is to be obtained, is therefore of the order of twentyfold.

Analysis of minerals—

Ground samples of dolomite and limestone (from I.C.I. Ltd., Lime Division, Buxton) that had been chemically analysed were examined chromatographically.

A 0·2 to 0·4-g sample in 4 ml of water containing a two-to-three-fold excess of analytical-reagent grade formic acid was warmed in a 10-ml Pyrex-glass beaker on a steam-bath until effervescence ceased. A dark brown residue of ferric oxide remained. The liquid was transferred to a 10-ml calibrated flask and made up to the mark with water. Aliquots of this solution were taken for chromatographic analysis.

The results were as follows—

	Dolomite		Limestone	
	Calcium found, %	Magnesium found, %	Calcium found, %	Magnesium found, %
Chemical analysis	31·2	5·8	38·8	0·42
Chromatographic analysis	32·6	5·7	39·5	0·41

Good agreement was obtained between the chemical and chromatographic-analysis values. An empirical correction factor was not applied to the chromatographic calcium values, which are therefore slightly high. The total amount of silica, iron and aluminium impurities is about 2 per cent., *viz.*, dolomite, 2·16 per cent.; limestone, 1·69 per cent. (on analysis dolomite contained: calcium carbonate, 77·81 per cent.; magnesium carbonate, 20·03 per cent. Similarly limestone contained: calcium carbonate, 96·85 per cent.; magnesium carbonate, 1·46 per cent.). Most of the impurities remained undissolved after the treatment with formic acid. A very slight trace of iron dissolved and was detected on the chromatogram, but this did not interfere with the spectrophotometric determinations.

SEPARATIONS ON A CELLULOSE COLUMN—

Solutions of 5 to 10-mg quantities of the formates of the alkaline-earth metals in 0·5 ml of water were eluted on a column (100 cm long × 1·4 cm in diameter) of Whatman Ashless Cellulose, according to the technique of Pollard, McOmie and Stevens.[21] Barium, strontium and calcium are quantitatively separated, but magnesium is only partly separated from calcium. On a paper chromatogram, magnesium moves in a water-impoverished zone. Such a zone is not present on a column, since it is displaced by the passage of solvent before the column is used, and hence the separation of magnesium from calcium is altered.

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The Determination of Sulphate in Thorium Nitrate

BY J. CLINCH

A new method utilising ion-exchange resin is proposed for the determination of sulphate in thorium nitrate. Results obtained with this method show that it is possible to determine sulphate in thorium nitrate with a coefficient of variation of about 2 per cent. Recovery of added sulphate in thorium nitrate appears to be complete within experimental error.

THORIUM nitrate manufactured for the gas-mantle industry contains from 1 to 2 per cent. of thorium sulphate. This is a deliberate addition and facilitates the production of a porous oxide on ignition. Purer grades, primarily for analytical use, are also made and they contain a maximum of about 0·1 per cent. of sulphur trioxide. In order to assess the efficiencies of various processes for the purification of thorium nitrate it is necessary to be able to determine the sulphate content of thorium nitrate with a reasonable degree of accuracy. The accurate determination of the sulphate content is, however, not possible by standard methods for, if a solution of a barium salt is added to a solution of thorium nitrate containing sulphate, precipitation of insoluble barium sulphate is very slow and rarely complete.[1] The precipitate formed contains a large amount of adsorbed thorium[2] and nitrate[3] ions and the method cannot be used for quantitative determinations.

Early workers[4,5] were aware of the necessity for the removal of thorium before determination of the sulphate content and their methods have been examined. This investigation showed that the older methods were generally unreliable and a new method is therefore proposed.

Although it was generally realised that thorium had to be removed before sulphate could be determined, it was apparently not realised that the nitrate ion had also to be removed in order to avoid its strong occlusion on the barium sulphate precipitate. White[4] removed thorium by fusion of the nitrate with four times its weight of potassium hydroxide. After leaching and filtration, sulphate was determined in the filtrate in the usual way. Some objections to the method are that if the nitrate under examination has only a small sulphate content a proportionately larger sample must be taken and the bulk of the melt becomes inconveniently large. The method also has the disadvantage that it not only retains the nitrate ion in the final solution but also introduces potassium ions, the presence of which is not advisable in the precipitation of barium sulphate.[3] This method will not be considered further.

The method given by Meyer and Hauser[5] removes thorium by a single precipitation with the stoicheiometric amount of oxalic acid. After filtration a known fraction of the supernatant solution is taken for determination with barium chloride. No attempt is made to remove the nitric acid formed in the reaction or to control the acidity of the barium sulphate precipitation. A thorium nitrate found to contain 0·82 per cent. of sulphur trioxide by the procedures to be outlined gave a result of 1·06 per cent. of sulphur trioxide by Meyer and Hauser's method.

A similar method is proposed by Rosin,[6] who evaporates the nitrate twice with concentrated hydrochloric acid and removes thorium as the oxalate with a 200 per cent. excess of oxalic acid.

The oxalate method of Meyer and Hauser has, however, proved useful for routine work, as it requires no special equipment or skill. The determination has been improved by removal of the nitrate ion and adjustment of the acidity by evaporation to dryness with concentrated hydrochloric acid. A 0·5-g portion of sodium carbonate is added to the solution before evaporation to fix the sulphate ion.

A new method involving the use of ion-exchange resins is now proposed. The sample is dissolved in water and passed down a column of cation-exchange resin in the hydrogen form. The eluate is collected and evaporated to dryness with concentrated hydrochloric acid and 0·5 g of sodium carbonate. Details of this method are given later. Experiments were performed to determine the minimum amount of resin necessary to remove the thorium from 10 g of thorium nitrate. This must be effected, as any thorium remaining will co-precipitate with the barium sulphate precipitate.[2] Increasing the amount of resin improves the removal of thorium, as shown in Table I.

TABLE I

REMOVAL OF THORIUM BY RESIN

Sample weight of thorium nitrate = 10 g

Weight of resin, g	16	32	48	64
Thorium nitrate unabsorbed, %	24	0·5	0·04	0·001

The sulphate content of the solution was finally determined with barium chloride. The literature on this determination is very contradictory. The method described for precipitating barium sulphate (p. 360) was therefore adopted and the results were examined.

METHOD INVOLVING PRECIPITATION WITH OXALIC ACID

REAGENTS—

Hydrochloric acid, concentrated, 11·4 N.
Oxalic acid, crystalline.

PROCEDURE—

Dissolve 10 g of sample in 250 ml of water and add 5 ml of concentrated hydrochloric acid. Transfer to a 500-ml calibrated flask and dilute to within about 50 ml of the mark. Add 5·0 g of oxalic acid, shaking until the acid has dissolved, and then heat the flask in a boiling-water bath for 2 hours. Cool the flask to room temperature, make the contents up to the mark, shake well and allow the precipitated thorium oxalate to settle.

By means of a clean dry rubber tube withdraw the clear supernatant solution and filter through a dry Whatman No. 540 filter-paper until exactly 400 ml (equivalent to 8 g of sample) are obtained. Use this solution for the determination of sulphate (see "Method for precipitating barium sulphate," p. 360).

Method involving separation by ion exchange

Reagents—

Ion-exchange resin—Zeo-Karb 225(H), 30 to 80 mesh, damp as supplied.

Hydrochloric acid, dilute, 3·4 N—Dilute 60 ml of concentrated hydrochloric acid to 200 ml with water.

Procedure—

Take a glass tube about 16 mm in diameter and about 40 cm long. Place at the bottom a glass-wool plug, about 15 mm thick, supported on a rubber bung with a glass-tube outlet having a screw-clip to control the rate of flow of liquid. Add 64 g of resin to the tube, and back wash with water to remove air bubbles. Allow the resin to settle and drain to the top of the bed. Add 115 ml of dilute hydrochloric acid (3·4 *N*) to the top of the column and allow to pass through in 15 minutes. Wash the column during 20 minutes with 100 ml of water and allow to drain to bed level. Add 10 g of thorium nitrate sample to the column and wash through with water, collecting 400 ml of the eluant in a 600-ml beaker. Use this eluant for the determination of sulphate (see below).

Method for precipitating barium sulphate

Reagents—

Hydrochloric acid, concentrated, 11·4 N.

Barium chloride, 10 *per cent. w/v solution*—Dissolve 10 g of hydrated barium chloride in sufficient water to make 100 ml of solution.

Hydrochloric acid, diluted (1 + 50)—Mix 2 ml of concentrated hydrochloric acid with 100 ml of water.

Sodium carbonate, anhydrous.

Procedure—

Evaporate the filtrate from the oxalic acid precipitation or the eluate from the column almost to dryness with 0·5 g of sodium carbonate. Add 10 ml of concentrated hydrochloric acid and repeat the evaporation almost to dryness. Add 10 ml of concentrated hydrochloric acid and evaporate again. Add 2 ml of concentrated hydrochloric acid and dilute to 400 ml with water. Boil the solution and slowly add 25 ml of boiling barium chloride solution with constant stirring. Keep the solution hot for 1 hour and allow to cool overnight. Collect the precipitate on a 15-cm Whatman No. 540 filter-paper, washing it five times with 20-ml portions of diluted hydrochloric acid (1 + 50), and ignite it in a porcelain or platinum crucible at 900° C to constant weight. Weigh the residue as barium sulphate. Calculate the percentage of sulphate in the sample.

Results

A sample of thorium nitrate was taken, ground and mixed thoroughly. This was used for repeated determinations of the sulphate content by the two methods. The ion-exchange method was varied by using 48 g, 64 g and 80 g of resin. The preliminary washing with hydrochloric acid was varied in proportion. The results are shown in Table II.

Table II

Comparison of results from oxalic acid and ion-exchange methods

	Oxalic acid method	Ion-exchange method		
Weight of resin used	—	48 g	64 g	80 g
Number of determinations ..	10	10	10	8
Barium sulphate, g (mean) ..	0·1005	0·1363	0·1335	0·1337
Standard deviation	0·0031	0·0029	0·0029	0·0033
Sulphur trioxide, % (mean) ..	0·431	0·468	0·458	0·459
Standard deviation, %	0·013	0·010	0·010	0·011

These results, analysed statistically, indicate that—

(*a*) the oxalic acid method gives lower results than the ion-exchange method,

(*b*) the ion-exchange method gives low results if insufficient resin is employed (64 g or more of resin are required), and

(*c*) the precision of the ion-exchange method is sensibly the same as that of the oxalic acid method.

The low result from the oxalic acid method is probably due to co-precipitation of sulphate with the oxalate precipitate in the separation of thorium.

In order to determine whether the method applied over a range of sulphate concentration, a uniform sample of recrystallised thorium nitrate was mixed with various amounts of a standard sodium sulphate solution and analysed by the ion-exchange procedure. The results are shown in Table III.

TABLE III

DETERMINATION BY ION-EXCHANGE PROCEDURE WITH INCREASING SULPHATE CONTENT

Sulphur trioxide added, %	Sulphur trioxide found, %	Difference
nil	0·099	+ 0·099
0·141	0·202	+ 0·061
0·282	0·348	+ 0·066
0·423	0·485	+ 0·063
0·564	0·634	+ 0·070
0·705	0·766	+ 0·061
0·845	0·918	+ 0·073
0·986	1·039	+ 0·053
1·127	1·204	+ 0·077
1·268	1·332	+ 0·064
1·409	1·489	+ 0·080

Statistical examination of these results shows complete recovery, within experimental error, of added sulphate. The positive difference shown in Table III indicates that the recrystallised thorium nitrate used contained sulphate. The best estimate of the amount of sulphate present is the simple average of these differences, *i.e.*, 0·070 per cent. of sulphur trioxide.

Examination was also made of the barium sulphate precipitation under similar conditions to the method already given and the results were examined statistically. Precipitation of barium sulphate from solutions containing a constant amount of sulphate ion gave the following results: 0·1102, 0·1225, 0·1234, 0·1251, 0·1256, 0·1232, 0·1247, 0·1233, 0·1181 and 0·1201 g of barium sulphate. A theoretical result of 0·1233 g of barium sulphate was expected. The initial result of 0·1102 g appears to be out of series and statistical examination confirmed this view. After rejection of this result a mean estimate of 0·1229 g of barium sulphate with a standard deviation of $\pm$ 0·0024 g was obtained, in excellent agreement with the theoretically expected result of 0·1233 g of barium sulphate.

Determinations of various amounts of sulphate within a similar range to that used in Table III were also carried out and recovery was found to be complete within experimental error.

CONCLUSIONS

The elution of sulphuric acid from gas-mantle grade thorium nitrate has been shown to be feasible and to lead to a method for the determination of sulphate in thorium nitrate. The determination is an improvement on existing published methods.

The method has a coefficient of variation of about 2 per cent. and recovery of added sulphate appears to be complete within experimental error.

An examination has also been made of the errors attending the precipitation of barium sulphate and the difficulties previously reported of obtaining a correct result are confirmed. Hillebrand and Lundell[7] point out the principal objection that the weight of precipitate used for co-precipitation and solubility experiments is generally large and of the order of 2 g of barium sulphate. It is not known whether the errors attending the precipitation of this amount of barium sulphate are comparable with the errors when 0·2 g of barium sulphate is used.

I am grateful to the Directors of Thorium Limited for permission to publish this paper and to Mr. E. A. Simpson for the experimental analyses.

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The Routine Determination of Admixed Chalk in Flour

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Two methods for checking the chalk content of flour are described. The first method is colorimetric and based on the use of chloranilic acid; the second is based on the use of the flame photometer. They are conveniently applicable to the routine examination of large numbers of individual samples. The results obtained by both methods on a series of samples are compared with those yielded by a gasometric technique previously described.

Various methods for the determination of the compulsory addition of calcium carbonate ("creta") to flour have been described.[1,2,3] The two methods described below permit batches of up to fifty samples to be examined and, although the content of a single sample could be determined by these methods, their inherent design renders them less suitable than other established techniques. They have been designed for their convenience in routine control, and tedious operations such as ashing and filtration have been avoided.

CHLORANILIC ACID METHOD

The use of chloranilic acid (2:5-dichloro-3:6-dihydroxy-1:4-benzoquinone) for the determination of calcium has been described elsewhere.[4,5] It forms a sparingly soluble calcium salt in faintly acid solution, but many ions have been shown to interfere.[6,7] However, its application to flour fortified with calcium carbonate can be regarded as a special instance in which the concentration of the interfering ions contributed by the flour remains constant and relatively small compared with the calcium content. Preliminary investigation showed that the admixed calcium carbonate could be extracted from the flour by the use of an acid buffer solution. Upon the addition of chloranilic acid solution, the calcium salt separated out and the diminution of the colour varied directly with the calcium concentration. These two operations were then combined by preparing a solution of chloranilic acid in the buffer reagent. The absorptiometric determination of the diminution of the chloranilic acid colour at this stage was complicated by one factor. Under such conditions the supernatant liquid always remained slightly turbid, and this residual turbidity could not be removed by clarifying treatment without interference with the colorimetric determination. Absorptiometric readings were corrected for the error introduced by this factor by taking a second reading after the addition of sodium dithionite solution to decolourise the residual chloranilic acid.

Method

Reagents—

Standard calcium solution—Dissolve 8·3626 g of analytical-reagent grade calcium carbonate in the minimum quantity of acetic acid and dilute to 250 ml.

0·1 ml of this solution ≡ 5 oz of $CaCO_3$ per sack when 3 g of flour are taken.

Buffer solution, pH 4·0—Mix equal volumes of 0·2 *M* sodium acetate and 0·8 *M* acetic acid.

Chloranilic acid solution, 0·1 per cent.—Dissolve 1 g of chloranilic acid in 1 litre of hot buffer solution; set the solution aside overnight and then filter it.

Sodium dithionite solution—Dissolve 15 g of sodium dithionite, $Na_2S_2O_4$, in 100 ml of water. This solution should be prepared immediately before use.

PROCEDURE—

Dispense 40 ml of 0·1 per cent. chloranilic acid into each of a series of 100-ml conical flasks. One flask is required for each sample and ten for a series of standards. Set up standards at five concentration levels in duplicate by adding 0·0, 0·1, 0·2, 0·3 and 0·4 ml of standard calcium solution. Prepare test extracts by weighing 3·00 g of each sample into a flask.

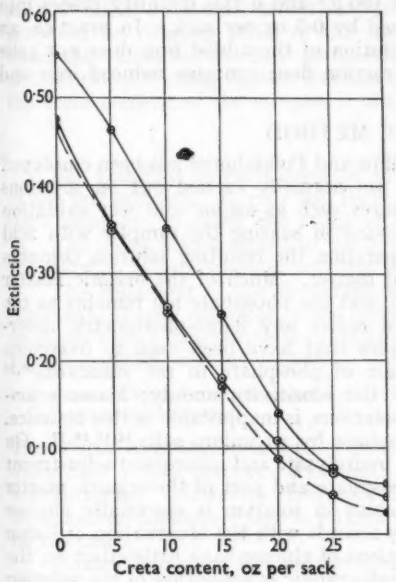

Fig. 1. Typical curves for various flours by the chloranilic acid method—
—⊕—⊕—, 70 per cent. extraction flour;
—⊗—⊗—, 80 per cent. extraction flour;
—⊙—⊙—, 90 per cent. extraction flour

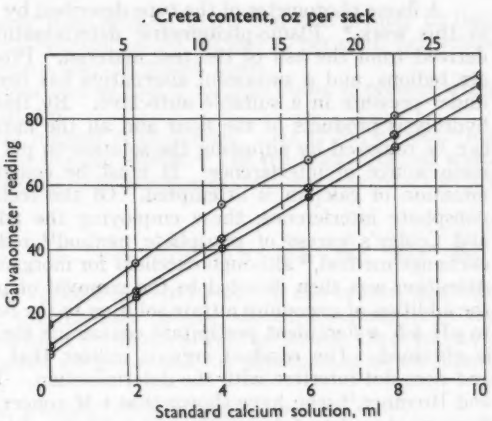

Fig. 2. Typical curves for various flours by the flame-photometric method—
—⊕—⊕—, 70 per cent. extraction flour;
—⊗—⊗—, 80 per cent. extraction flour;
—⊙—⊙—, 90 per cent. extraction flour

Close the flasks with rubber stoppers and mix the contents by vigorous shaking. Place the flasks on a suitable shaking apparatus and agitate gently for 30 minutes, taking care to loosen the stoppers at intervals to release the gas pressure. Set the flasks aside overnight in a cool place.

By means of a pipette fitted with a rubber bulb, remove 10 ml of the supernatant liquor, transfer it to a ½-inch colorimeter tube and read the extinction, E_1, at 540 mμ, using water as a reference solution. Decolourise by adding one drop of sodium dithionite solution and inverting two or three times and again determine the extinction, E_2. This second reading is a measure of the turbidity of the extract, and the difference of the readings, $E_1 - E_2$, is the extinction due to the colour of the solution.

Construct a graph relating the readings $E_1 - E_2$ obtained on the standard extracts to the calcium added. Under the conditions specified, 0·1 ml of standard calcium solution is equivalent to 5 oz of calcium carbonate per sack of flour. From this graph the calcium fortification of the samples can be read directly.

DISCUSSION

Fig. 1 shows typical curves obtained by this method for flours of 70, 80 and 90 per cent. extraction. Over the range of 0 to 20 oz per sack these graphs are essentially linear. The

extraction rate of flour under examination governs the relative displacements of these graphs and these displacements are associated with the natural contents of calcium and iron, as is shown by the following analysis of typical creta-free flours—

Declared extraction rate, % ..	70	80	90
Calcium, mg per 100 g	14	27	28
Iron, mg per 100 g	0·5	1·5	3·0

Standard extracts should be prepared from flour of an extraction rate similar to that of the samples under examination. In this way, the concentration of ions extracted from the flour, which may interfere with the determination, is maintained at a similar level in the standard and test solutions. Iron, however, is a compulsory addition to low-extraction flour and its presence could introduce a minor error. The mean increase of iron content of fortified flours has been shown to be 0·96 mg per 100 g,[8] and if this quantity passes into solution, the apparent creta content will be depressed by 0·5 oz per sack. In practice, an error of this order is not encountered; complete solution of the added iron does not take place, as the "master-mix" used to fortify low-extraction flour contains reduced iron and not a water-soluble iron salt.

FLAME-PHOTOMETRIC METHOD

A flame photometer of the type described by Collins and Polkinhorne has been employed in this work.[9] Flame-photometric determinations are normally carried out on solutions derived from the ash of the test material. Procedures such as ashing and wet oxidation are tedious, and a successful alternative has been found in heating the samples with acid under pressure in a suitable autoclave. By this operation the resulting solution contains hydrolysis products of the flour and all the mineral matter. Much of the organic matter can be removed by adjusting the solution to pH 4·5, and the phosphate ion remains as the main source of interference. It must be controlled before any flame-photometric determination of calcium is attempted. Of the techniques that have been used to overcome phosphate interference, those employing the addition of phosphate to the standards[10,11] and Cooley's excess of phosphate method[12] reduce the sensitivity unduly; Mason's ion-exchange method,[13] although excellent for inorganic solutions, is inapplicable in this instance. Attention was then directed to the removal of phosphate by zirconium salts.[14,15,16,17] On the addition of zirconium nitrate solution to the flour hydrolysate and subsequent adjustment to pH 4·5, a flocculent precipitate containing the phosphate and part of the organic matter is obtained. The residual organic matter that remains in solution is essentially glucose and does not interfere with the determination. This accords with the observation of Caton and Bremner,[18] who have shown that 4 *M* concentrations of glucose have little effect on the flame-photometric determination of calcium. Chromatographic examination of the solution obtained in these conditions showed that glucose was present without any significant quantity of other soluble carbohydrate material.

Control solutions for standardisation that simulate the composition of the test solutions are prepared from creta-free flour and standard calcium solution and treated in the same way as the other samples.

METHOD

REAGENTS—

Hydrolysis acid—Mix 100 ml of concentrated hydrochloric acid with 25 ml of acetic acid and dilute with distilled water to 4 litres.

Zirconium nitrate solution—Dissolve 20 g of zirconium nitrate, $ZrO(NO_3)_2.2H_2O$, in 800 ml of water containing 5 ml of concentrated nitric acid and warm to dissolve. Filter and make up to 1 litre.

Ammonia solution—Dilute 250 ml of concentrated ammonia solution, sp.gr. 0·880, with 650 ml of water.

Bromocresol green—A 0·5 per cent. aqueous solution of bromocresol green.

Standard calcium solution—Dissolve 1·2485 g of analytical-reagent grade calcium carbonate in distilled water containing 4 ml of concentrated nitric acid and dilute to one litre. 1 ml of this solution contains 0·5 mg of calcium.

PROCEDURE—

Weigh out 2·00 g of each sample into a 150-ml beaker, add 40 ml of hydrolysis acid, stir well and cover with a watch-glass. Transfer the beaker to an autoclave and heat for

20 minutes at a pressure of 20 lb per sq. inch. Allow to cool, and add 2 ml of zirconium nitrate solution and sufficient ammonia solution (about 10 ml) to make the pH value 4·5, using bromocresol green as an external indicator.

Transfer to a 100-ml calibrated flask, dilute to the mark with distilled water and mix. Set the solution aside overnight, and then decant approximately 10 ml for measurement by means of the flame photometer.

Take six 2-g portions of a creta-free flour of extraction rate similar to that of the samples and add 0, 2, 4, 6, 8 and 10 ml of standard calcium solution. Submit these to exactly the same treatment as that given to the samples. These standards are equivalent to 0, 5·6, 11·2, 16·8, 22·4 and 28·0 oz of creta per sack.

Set the zero of the flame photometer with distilled water and adjust the sensitivity control so that a full scale deflection ("100") is obtained with a calcium standard (50 μg per ml) prepared by diluting 10 ml of the standard calcium solution (0·5 mg per ml) to 100 ml. Read the values for the flour standards and the samples, and from the former plot a graph of instrument reading against calcium content, expressed as oz per sack. From this graph, the creta content of the samples is obtained directly. Typical graphs are shown in Fig. 2.

Discussion

The standard extracts must be prepared from flour of extraction rate similar to that of the test samples. In practice, three creta-free flours of 70, 80 and 90 per cent. extraction were adequate to cover the range of commercially produced flours.

The acetic acid is added to the hydrolysis acid so that a suitable buffer is present when the solution is subsequently adjusted to pH 4·5.

The behaviour of the zirconium nitrate reagent is of interest. Its action in removing phosphate is only complete in the presence of organic matter. The readings obtained when an 80 per cent. extraction flour containing the statutory addition of creta was treated according to this method were as follows—

Zirconium nitrate solution added, ml ..	0	1	2	3	4	5	6	8	10
Galvanometer deflection	19	49	52·5	53·5	54·5	56·0	58·5	58·5	59·0

The volume of zirconium nitrate solution was varied from 0 to 10 ml. It can be seen that with quantities of this reagent exceeding 2 ml, the readings become almost independent of the volume added. However, when more than 4 ml of zirconium nitrate are added, the precipitate of zirconium hydroxide that is formed does not settle out and the supernatant liquid cannot be decanted.

From these observations the optimal addition of this reagent is 2 to 4 ml.

The repetitive addition of fixed quantities of reagents, which these methods entail, is facilitated by the use of a suitable dispensing apparatus.[19]

Results

Initially two flour samples (L and H) containing 9 and 21 oz of creta per sack were selected and a third sample (M) was prepared by intimately mixing equal weights of each. These three samples (L, M and H), together with a creta-free "National" flour, were analysed by the methods described, and by a gasometric technique.[1] A further portion of each sample was ashed and the total calcium was determined by oxalate precipitation and permanganate titration. The results obtained are shown in Table I.

Table I

Creta contents of flour samples by various methods

Sample	Creta content by chloranilic acid method, oz per sack	Creta content by flame-photometric method, oz per sack	Creta content by gasometric method, oz per sack	Creta content by ashing and oxalate method,* oz per sack
L	9·5	9·1	8·3	11·2 (9·0)
M	14·9	14·9	14·9	16·8 (14·6)
H	21·2	20·6	21·9	23·5 (21·3)
Creta-free	0	0	0	2·2 (0)

* The values in brackets are obtained by subtracting the "natural" calcium of the creta-free sample from the total calcium content.

A series of "standard" flours of known creta content was prepared by the addition of weighed quantities of calcium carbonate to creta-free flour of 70, 80 and 90 per cent. extraction to give contents equivalent to 0, 3·5, 7·0, 10·5 .. to 28 oz per sack. The values obtained by three methods on these prepared flours are shown in Table II.

TABLE II

CRETA CONTENT OF FLOUR SAMPLES BY VARIOUS METHODS

	Creta content by colorimetric method, oz per sack	Creta content by flame-photometric method, oz per sack	Creta content by gasometric method, oz per sack
70 per cent. extraction flour	3·5	3·5	3·7
	7·2	7·0	7·1
	10·6	10·6	10·6
	13·6	14·1	13·9
	16·7	17·4	17·2
	19·2	20·3	21·3
	22·0	23·4	23·9
	29·0	28·1	28·8
80 per cent. extraction flour	3·7	3·9	3·4
	7·2	6·7	6·9
	10·7	11·1	10·6
	14·0	13·8	14·2
	17·4	17·7	17·9
	20·7	20·5	20·8
	24·1	24·5	24·7
	27·9	28·4	29·0
90 per cent. extraction flour	3·8	3·8	3·5
	6·8	7·3	7·0
	9·7	11·1	10·1
	13·6	13·6	13·7
	16·9	17·4	17·7
	19·8	20·5	20·5
	23·4	24·2	24·7
	27·0	28·0	28·4

We are indebted to the Government Chemist for permission to publish this work.

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Notes

THE DIRECT ANALYSIS OF URANIUM - GALLIUM ALLOYS

PREVIOUS reports from this laboratory have described the determination of gallium in the presence of uranium. The separation of the gallium was necessary, however, before its determination could be completed either gravimetrically[1] or volumetrically.[2] The volumetric procedure with ethylenediaminetetra-acetic acid (EDTA) is extremely rapid and is preferred for the determination of the separated gallium. Recently, Milner and Edwards[3] developed a procedure involving the use of EDTA for the direct determination of zirconium in uranium - zirconium binary alloys. An excess of EDTA is added to form a complex with the zirconium in solutions of pH 2·2 and the unused reagent is determined by titration with a standard ferric iron solution, benzohydroxamate being used as the indicator. Uranium does not form a complex with the EDTA at this pH value, and interference from the uranyl ions in the visual detection of the end-point is overcome by using the absorptiometric method of end-point determination. From the results of this work a similar procedure appeared feasible for the direct determination of the gallium content of uranium - gallium alloys, since a fairly strong complex is formed by this element with EDTA in solutions at pH 2. Details of the procedure developed for this determination are given in this Note. A volumetric procedure was found to be the most convenient for the determination of the uranium content of these alloys. The uranium is reduced to the quadrivalent state by passing a hydrochloric acid solution of the sample through a lead reductor and is then determined by titrating with a standard ceric solution. There is negligible interference from the gallium in the uranium determination.

METHOD FOR DETERMINING GALLIUM

REAGENTS—

Standard iron solution, 0·1 M—Dissolve 5·585 g of Specpure iron in 20 ml of hydrochloric acid, sp.gr. 1·16, and then oxidise the ferrous iron by the dropwise addition of nitric acid, sp.gr. 1·42. Dilute this solution to 1 litre with water. Suitably dilute an aliquot of this solution to give a 0·02 *M* solution.

EDTA solution, 0·1 M—Dissolve 37·23 g of the disodium salt of EDTA in water and dilute to 1 litre. Standardise this solution against a standard 0·1 *M* zinc solution prepared from Specpure metal, using Eriochrome black T as the indicator.[4] Suitably dilute an aliquot of this solution to give a 0·02 *M* solution.

Indicator solution—Dissolve 2·5 g of potassium benzohydroxamate in 100 ml of water.

PROCEDURE—

Transfer the slightly acidic gallium solution to a squat beaker and dilute to a volume of about 300 ml. Add sufficient of the 0·02 *M* EDTA solution to form a complex with the gallium and leave a slight excess of the reagent. Then adjust the pH to a value of 2·2, as shown by a direct-reading pH meter, by the careful addition of dilute ammonia solution. Add 1 ml of the indicator solution and position the beaker in an E.E.L. absorptiometer (Evans Electroselenium Ltd.). Adjust a motor-driven stirrer to mix the solution mechanically. Then adjust the absorptiometer to give zero reading with Ilford No. 605 filters in place and do not alter this setting during the titration. Make successive additions of the 0·02 *M* ferric iron solution from a burette with the tip touching the surface of the solution. Note the optical-density reading when the galvanometer needle has settled down after each addition of titrant. Plot a graph of the volume of iron solution added against optical-density reading and determine the end-point of the titration from the intersection of the two straight-line portions of the graph. Calculate the gallium content from the expression—

$$\text{Gallium, mg} = 69{\cdot}72\, M_2(V_2 - V_1 M_1/M_2),$$

where M_1 = molarity of ferric iron solution,
V_1 = volume of ferric iron solution,
M_2 = molarity of EDTA solution, and
V_2 = volume of EDTA solution.

RESULTS—

The above procedure was tested by applying it to aliquots of a standard solution of gallium in hydrochloric acid, both in the presence and in the absence of uranium. The results obtained

were satisfactory and are given in Table I. From these results it is seen that there is negligible interference from uranium, and the procedure should be applicable to the analysis of uranium - gallium alloys covering a wide range of composition.

TABLE I

DIRECT DETERMINATION OF GALLIUM IN THE PRESENCE OF URANIUM

Weight of gallium taken, mg	Weight of uranium taken, mg	Weight of gallium found by titration, mg	Difference mg
2·80	—	2·80	nil
7·02	—	6·98	− 0·04
14·04	—	14·06	+ 0·02
21·06	—	21·05	− 0·01
28·08	—	28·06	− 0·02
14·04	20	14·00	− 0·04
14·04	100	14·06	+ 0·02
14·04	200	14·02	− 0·02
14·04	500	14·09	+ 0·05
14·04	1000	14·10	+ 0·06
14·04	2000	14·13	+ 0·09

DETERMINATION OF URANIUM

Macro amounts of uranium are most conveniently determined volumetrically after reduction to the quadrivalent state. The zinc-amalgam reductor is generally recommended for this determination, the uranium being reduced to a mixture of the tervalent and quadrivalent states. It is necessary to pass air through the solution to convert the tervalent uranium to the quadrivalent form before proceeding with the titration. More recently the lead reductor has been suggested for this determination, and a critical examination of this reductor has been carried out by Bacon and Milner.[5] These workers found it very suitable for the accurate determination of the uranium content of aluminium - uranium binary alloys. The main advantages of this reductor are that it is easily prepared and that it reduces the uranium to the quadrivalent state only. This procedure should be applicable to the analysis of uranium - gallium alloys provided that the gallium does not cause interference. Therefore, the behaviour of gallium in the uranium determination was studied by carrying out experiments with the apparatus and procedure previously reported.[5] Suitable artificial solutions were prepared from standard solutions of uranium and of gallium in hydrochloric acid and the volumes were adjusted to almost 60 ml with water. Then after the addition of 20 ml of hydrochloric acid, sp.gr. 1·18, each solution was passed through the reductor, followed by suitable washing with *N* hydrochloric acid. The uranium content was finally determined by titration against a standardised ceric solution, ferroin being used as the indicator. Results obtained on solutions containing 119·1 mg of uranium in the presence of various amounts of gallium are given in Table II, from which it can be seen that the gallium has only a small interference.

TABLE II

DETERMINATION OF URANIUM IN THE PRESENCE OF GALLIUM

Uranium taken, mg	Gallium taken, mg	Uranium found by titration, mg	Difference, mg
119·1	nil	119·1	nil
119·1	25	118·9	− 0·2
119·1	100	119·4	+ 0·3
119·1	250	119·6	+ 0·5
119·1	500	119·7	+ 0·6
119·1	1500	119·6	+ 0·5

ANALYSIS OF ALLOY SAMPLES

Nitric acid is the best acid for dissolving alloy samples, but unfortunately the lead-reductor method for uranium is not applicable to solutions containing nitrate. Alloy solutions were therefore evaporated to fumes of sulphuric acid and fumed for a sufficient time to ensure the removal of nitric acid. The procedure adopted consisted in dissolving 2 g of alloy in nitric acid, adding 20 ml of concentrated sulphuric acid and then evaporating to fumes of this acid. After cooling, the solution was diluted slightly with water and reheated to fumes of sulphuric acid. The sample

solution was then diluted to a volume of 100 ml with water and separate aliquots were taken for the gallium and uranium determinations. The uranium determination was carried out first so that an indication of the gallium content could be obtained by difference. Then a calculated amount of EDTA was added to the aliquot for the gallium determination and the titration with the standard iron solution was carried out as described earlier. Results obtained for typical alloy samples are given in Table III.

TABLE III

ANALYSIS OF URANIUM - GALLIUM ALLOY SAMPLES

Sample No.	Composition determined by analysis		
	Uranium, %	Gallium, %	Total, %
1	21·9	78·4	100·3
2	46·0	54·4	100·4
3	91·2	8·6	99·8
4	46·6	53·6	100·2

CONCLUSIONS

The procedures described above should be suitable for the routine analysis of uranium - gallium alloy samples, since chemical separations are avoided and both constituents are determined by volumetric methods.

Thanks are due to Mr. G. W. Sneddon for assistance with the experimental work.

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DETERMINATION OF SMALL QUANTITIES OF NICKEL WITH α-FURILDIOXIME

THE determination of microgram amounts of nickel by oxidation of the nickel^{II} - dimethylglyoxime complex to a red compound is well known.[1] The method was applied in these laboratories to aqueous samples containing plutonium and iron, but the results were erratic. The samples were extracted with cupferron to remove plutonium and iron, nickel remaining in the aqueous phase. On wet destruction of the cupferron, addition of dimethylglyoxime and alkaline oxidation with bromine, the red nickel complex that developed was often unstable.

Alternative methods for the separation and determination of nickel at low levels were examined. The direct colorimetric comparison of chloroform extracts of a nickel^{II} - α-dioxime complex suggested itself as a rapid and convenient procedure. The comparison of chloroform extracts of nickel^{II} - dimethylglyoxime for routine determination of nickel in uranium has been described.[2] I applied this technique successfully to samples of plutonium containing 100 μg of nickel or more.[3] Extractions were performed in alkaline solutions in the presence of sodium citrate to prevent precipitation of heavy metals, such as iron, uranium and plutonium. The colour was not sufficiently sensitive for our needs however.

Gillis, Hoste and van Moffaert[4] have described the use of *cyclo*heptanedionedioxime (heptoxime) as a sensitive colorimetric reagent for nickel. The complex is precipitated quantitatively from an aqueous solution at pH 3·8 to 11·7 and is soluble in chloroform to give a yellow solution with maximum absorption at 377 mμ and a molar extinction coefficient of 116,200. As heptoxime was not readily available, α-furildioxime was examined as a reagent for nickel under similar conditions. This reagent has been described for gravimetric and colorimetric determinations of nickel in aqueous solutions.[5] As far as is known, however, no quantitative results have been recorded for chloroform solutions of the nickel - α-furildioxime complex.

The present work was carried out in order to develop a sensitive and robust method for nickel at the 1 to 10-μg level. Interferences of iron, chromium and alkali-metal salts have been studied, and the method has been used for the determination of nickel in plutonium solutions.

Experimental

Preliminary investigations showed that α-furildioxime forms a red coloration or precipitate in alkaline chloride or sulphate solutions containing more than 5 μg of nickel per ml. Nitrate solutions were not studied. When the aqueous suspension was shaken with chloroform, the red colour disappeared and an intense yellow colour appeared in the chloroform layer. Examination of the chloroform solution with a Uvispek spectrophotometer showed an absorption maximum at 435 mμ, with a molar extinction coefficient of 15,400 (see Fig. 1). This band was shown to obey Beer's law over the range 1 to 20 μg of nickel. The effect of iron was then studied. It was found that ferrous iron gives an intense purple coloration or precipitate under the conditions

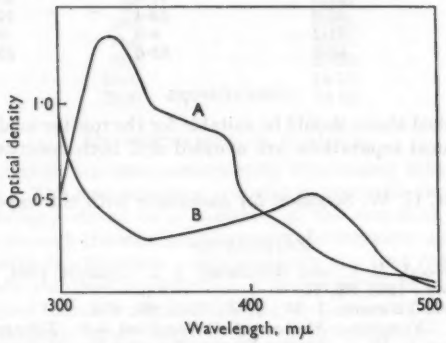

Fig. 1. Absorption spectra: curve A, nickel - dimethylglyoxime complex in chloroform (40 μg of nickel; volume, 10 ml; 4-cm cell; absorption maximum, 326 mμ; molecular extinction coefficient, 4940); curve B, nickel - α-furildioxime complex in chloroform (5 μg of nickel; volume, 10 ml; 4-cm cell; absorption maximum, 435 mμ; molecular extinction coefficient, 15,400)

for the formation of the nickel complex, and this seriously increases the optical density when more than 10 μg of iron are present. This effect may be eliminated by adding potassium dichromate and then sodium citrate to form a complex with the ferric iron produced. By this means at least 500 μg of iron may be present in a determination, and it was noted that up to 200 μg of chromium present as either chromium^{III} or chromium^{VI} do not interfere. The addition of sodium citrate also prevents precipitation of hydrolysable cations, such as uranium (as $UO_2^{\cdot\cdot}$) and plutonium, which would otherwise be precipitated under the alkaline conditions of extraction.

In the presence of alkali-metal salt solutions up to 0·1 *M*, the optical density of the extracted complex is not affected, but the rate of extraction is retarded and the time of agitation has to be increased.

Method

Reagents—

Potassium dichromate solution, N.

Sodium citrate—A 10 per cent. solution in water.

α-Furildioxime—A 1 per cent. w/v solution in 50 per cent. aqueous ethanol.

Ammonium hydroxide, sp.gr. 0·880.

Standard nickel solution—Dissolve 0·673 g of analytical-reagent grade nickel ammonium sulphate, $NiSO_4.(NH_4)_2SO_4.6H_2O$, in 100 ml of water, add 10 ml of 5 *M* sulphuric acid and dilute to 1 litre. This solution contains 100 μg of nickel per ml. Dilute 1 ml of this solution to 10 ml as required to give a solution containing 10 μg of nickel per ml.

Procedure—

Place an aliquot of sample solution containing from 0 to 20 μg of nickel, which should be only slightly acid (not less than pH 1), in a 50-ml separating funnel. Add 0·05 ml of *N* potassium

dichromate solution, 5 ml of sodium citrate solution, 0·06 ml of α-furildioxime solution and 0·5 ml of ammonium hydroxide. Mix and extract the solution with three 7-ml portions of chloroform, shaking the funnel 200 times by hand for each extraction. Combine the solvent extracts in a 25-ml calibrated flask and dilute with chloroform to the mark; measure the optical density of this solution immediately. Use a Spekker absorptiometer, with 4-cm cells and Ilford No. 601 filters. Perform a reagent blank at the same time.

Prepare a calibration graph by taking aliquots of the standard nickel solution equivalent to 5, 10, 15 and 20 μg of nickel, and carry out the procedure described above. Plot a graph of amount of nickel against optical density, and read the amount of nickel in the sample from this graph, after correcting the optical densities of sample and standard for the reagent blank.

The author acknowledges permission from the United Kingdom Atomic Energy Authority to publish this work.

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November 7th, 1955

NITROSORESORCINOL MONOMETHYL ETHER AS A REAGENT FOR IRON AND COBALT

Nitrosoresorcinol monomethyl ether (3-methoxy-5-nitrosophenol) has been prepared and examined by Heinrich and Rhodius,[1] but has not previously been used as a reagent.

Experimental

Preparation of the reagent—

The reagent is prepared by dissolving 12·5 g of *m*-methoxyphenol in 60 ml of industrial methylated spirit, adding 10 g of sodium nitrite in 30 ml of water and, while keeping the temperature of the mixture below 5° C and constantly stirring, adding 10 ml of hydrochloric acid, sp.gr. 1·18. The volume is increased to 250 ml by adding ice - water and, after 1 hour, the solid product is collected by filtration, suspended in water and steam-distilled, the distillate on filtration yielding golden needle-like crystals. This material is only slightly soluble in water, but is soluble in a variety of organic solvents.

Complexes formed by the reagent—

Ferrous iron produces a water-soluble green complex, detectable at extreme dilution, also soluble in *iso*amyl and *n*-butyl alcohols. Cobalt produces a red-brown complex, insoluble in water, but soluble in various alcohols and in benzene. This is also detectable at very high dilution. Ferric iron, copper and nickel produce brown complexes, but of such low sensitivity in analysis that iron and cobalt may usually be determined spectrophotometrically in their presence without error.

Determination of ferrous iron—

A solution of the reagent is made by dissolving 0·1 g in 25 ml of either glacial acetic acid or acetone; 0·5-ml amounts of this solution are added to 10-ml aliquots of suitable dilutions of ferrous iron solutions. It is found that the wavelength of maximum absorption is between 700 and 710 mμ, the pH value of the solution for maximum colour development being 2·0, but values between 1·5 and 2·5 introduce no significant error.

A straight-line calibration graph is obtained for ferrous iron by the use of a Spekker absorptiometer, tungsten-filament lamp illumination, H503 heat filters, Ilford No. 608 red filters, 1-cm

cells and a water setting of 1·0. The reagent alone gives no absorption reading. The colour formed reaches a maximum in under 10 minutes, and does not subsequently change in 2 hours, being stable for some days.

Although ferric iron produces an insoluble brown complex, the sensitivity of formation is such that a concentration of three times that of the ferrous iron can be tolerated for a short time. After longer periods of time than 10 minutes, the reducing action of the reagent becomes apparent. The ferric iron may be easily reduced by reaction with sulphur dioxide, most of the gas being removed by boiling, and the determination is then carried out. Nickel and copper both give nearly insoluble brown complexes with the reagent, but they are of low sensitivity and may be tolerated if present in concentrations of one-hundred and of ten times, respectively, that of the ferrous iron. If cobalt is present in concentrations greater than twice that of the ferrous iron, the solution must be filtered. Filtering or extraction methods to be described permit iron and cobalt to be determined in a material. Another procedure, in which the iron colour is destroyed by adding 2 ml of concentrated hydrochloric acid to 20 ml of solution, permits the absorption given by the cobalt complex, stable in strongly acid solution, to be subtracted from the total absorption to give the result for ferrous iron.

In a typical determination on a duralumin-type light alloy, the declared iron content of which was 0·50 per cent. (Analysed Standard), a 0·50-g sample was dissolved in aqueous sodium hydroxide. The solution was rapidly made acid with hydrochloric acid and diluted to 100 ml; a 10-ml portion was treated with sulphur dioxide, neutralised with ammonium carbonate, made just acid with acetic acid and diluted to 100 ml. Two 10-ml portions were treated with the reagent, one having the colour destroyed by hydrochloric acid and being used as a blank. The iron content of the alloy was found to be 0·52 per cent.

Determination of cobalt—

For cobalt, solvent-extraction methods are used. The optimum pH range for complex formation is 6 to 8, the maximum absorption occurring at 380 mμ. The sensitivity is high, 0·001 g of cobalt per litre giving a visible precipitate. After formation of the complex, the addition of hydrochloric or nitric acids has no effect on the complex.

A calibration graph is obtained by using a Spekker absorptiometer, with a tungsten-filament lamp, H503 heat filters, Ilford No. 604 green filters, 5-mm cells and a water - benzene setting of 1·0. The complex is dissolved by shaking each portion of solution, after addition of the reagent, with 10 ml of benzene and filtering the benzene extract.

In the presence of large excess of iron, for example, in a steel, the iron colour is destroyed by means of hydrochloric acid, the solution is extracted with benzene and the extract is washed with hydrochloric acid. The iron may subsequently be determined by neutralising the aqueous solution with ammonia solution, making acid with acetic acid, reducing with sulphur dioxide, if necessary, and diluting to a suitable volume, no further reagent being added.

If the alloy contains nickel, it is dissolved in aqua regia, and the solution is then suitably diluted and reduced with sulphur dioxide. After addition of the reagent, the solution is rendered strongly ammoniacal, when the iron and other interfering elements will remain in the aqueous phase after the cobalt has been extracted into the benzene, the benzene extract being washed with water and then dilute acid.

Discussion

I consider the reagent in some ways superior to existing reagents. Compared with 2:2′-dipyridyl, with which, among others, work was done by Feigl and Hamburg,[2] the colour of the nitrosoresorcinol monomethyl ether - ferrous iron complex is more suitable for spectrophotometric determination, spectrum filters being used, and the copper interference is not so serious.

Similar remarks apply to *o*-phenanthroline. The present reagent is similar in reactions to nitroso-R salt, but has the advantage that the cobalt complex is insoluble in water, but may be extracted with solvents. This, in my opinion, provides a simpler method of determining cobalt than the usual methods in which nitroso-R salt is used, such as that of Haywood and Wood.[3] In this method, complicated manipulations of acidity and buffering are necessary to eliminate the iron.

In my opinion, the reagent is superior to cupferron and 1-nitroso-2-naphthol for determining iron, in that the iron complex is soluble without addition of reagents such as ethyl acetate. Also there is much less serious interference by copper than with cupferron.

It may therefore be stated that the principal advantages claimed for the use of the present reagent arise from the solubility of the ferrous iron complex in water and its colour change with

acidity, the insolubility of the cobalt complex, and the ease of extraction by organic solvents. In addition, the colour reaction for iron has a very suitable absorption wavelength and is extremely sensitive.

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DETERMINATION OF SULPHIDE SULPHUR IN MINERALS

The methods so far suggested for the determination of sulphides involve oxidation of the sulphide to the corresponding sulphate and subsequent determination of the sulphate gravimetrically. Oxidation may be carried out either by digesting with fusion mixture or by the wet procedure, in which aqua regia or nitric acid and bromine[1] are used. It is clear that the oxidation methods can give the total sulphur including sulphide and sulphate. The presence or formation of an insoluble sulphate such as barium or lead sulphate restricts further the adaptability of this method. Moreover, the time required for the oxidation method is considerable.

The present investigation describes an accurate and rapid method for the determination of sulphide sulphur in presence of sulphates and other acid radicals; it is based on the interaction between hydriodic acid and metallic sulphides, use being made of this reaction by Rao[2] and by Murthy[3,4] for determining mercuric sulphide. The sulphides are reduced by hydriodic acid to hydrogen sulphide and the corresponding metal iodides form complexes with excess of hydrogen iodide and remain in solution. The liberated hydrogen sulphide can be removed with a current of nitrogen or hydrogen and absorbed by a suspension of cadmium hydroxide and determined iodimetrically.

Method

Reagents—

Hydriodic acid—Mix a 50 per cent. aqueous solution of potassium iodide with an equal volume of concentrated hydrochloric acid. Add a few crystals of sodium hypophosphite to reduce any liberated iodine. Decant the clear supernatant liquid from the precipitated potassium chloride.

Cadmium hydroxide suspension—Add 10 ml of *N* sodium hydoxide to 50 ml of 2 per cent. cadmium acetate solution.

Procedure—

Finely powder the mineral, taking care to avoid atmospheric oxidation. Weigh a suitable quantity (0·1 to 1 g) into a 50-ml round-bottomed flask having an interchangeable ground-glass neck into which a ground-glass connection with a dropping funnel and gas inlet and exit tubes are fitted.[2] Attach a wash bottle containing the suspension of cadmium hydroxide to the exit tube for absorption of hydrogen sulphide. Glass tubes with ground-glass joints or neoprene tubes should be employed in making the necessary connections. Lubricate the ground-glass joints with glycerol. The apparatus used in shown in Fig. 1.

Displace the air completely from the apparatus by means of a stream of hydrogen, and add the hydriodic acid mixture (5 to 10 ml) through the dropping funnel, continuing to pass the stream of hydrogen for about 1 hour with occasional shaking. The contents of the flask may be warmed if the dissolution of the sulphide is slow. Mix the contents of the wash bottle with 2 *N* acetic acid containing a known excess of standard iodine solution and titrate the unused iodine with standard sodium thiosulphate. It is necessary to have an excess of acetic acid (0·5 *N*) before titrating the residual iodine with sodium thiosulphate. The reaction between cadmium sulphide and iodine can also be hastened by the addition of 5 ml of dilute hydrochloric acid (2 *N*) for every 100 ml of the titrant. From the amount of iodine consumed, the sulphide equivalent can be calculated (when the amount of hydrogen sulphide is small, the diamine reagent[5] may be used). Typical results are indicated in Table I.

The results presented in Table I show that the new method for the analysis of galena gives a satisfactory value for sulphur, as is evidenced by the sulphur value for the sample of precipitated lead sulphide. With galena crystals, however, a consistent high value (0·5 per cent.) for sulphur is obtained by the wet-oxidation method, showing thereby the presence of a small quantity of

lead sulphate in the galena sample. This is also true of the carbonaceous galena, in which 3·5 per cent. of sulphur is present as lead sulphide and 1·88 per cent. as lead sulphate. With sphalerite the amount of oxidised sulphur is practically negligible (0·3 per cent. only).

The present method can also be conveniently employed for the determination of lead. The contents of the flask (after the hydrogen sulphide has been expelled) are filtered, and the residue is washed, ignited and examined for insoluble residue, including silica. The filtrate can be

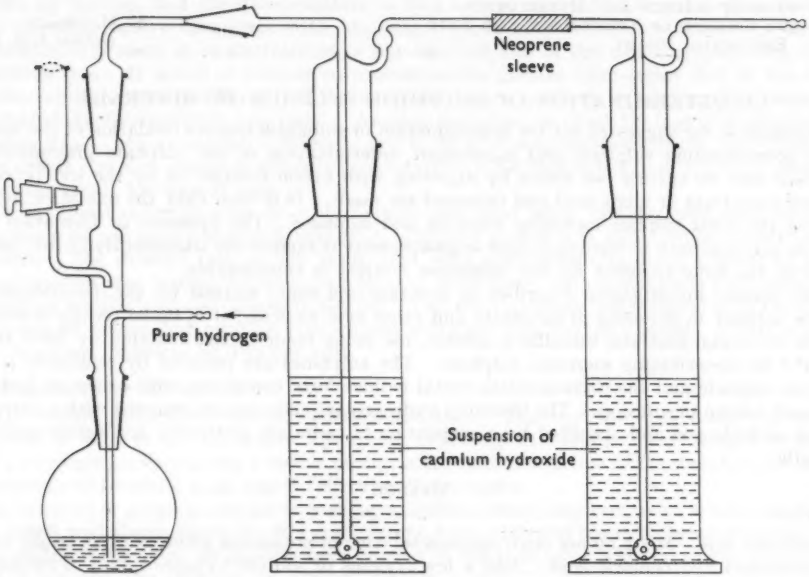

Fig. 1. Layout of apparatus

evaporated to dryness, digested with concentrated sulphuric acid and the lead determined as lead sulphate. The results for lead in Table I were obtained by the present method and show, for the precipitated lead sulphide, good agreement with the theoretical value. The value for galena is slightly lower than the theoretical value on account of the presence of lead sulphate, as pointed out already.

Results

Table I

Typical results for various sulphides

Sample	Sulphur found by the new method, %	Sulphur found by wet oxidation, %	Theoretical value for sulphur, %	Lead content, %	Theoretical value for lead, %
Galena crystals	13·22	13·94	13·38	86·16	86·70
Lead sulphide (precipitated) ..	13·32	13·33	13·38	86·72	86·70
Carbonaceous galena	3·54	5·42	—	63·12	—
Zinc blende (sphalerite)	8·68	8·98	—	6·95	—

As the aqueous solution of hydrogen iodide has the capacity to form complex iodides with various metal iodides, this method can further be extended to determine silver sulphide, nickel sulphide and cobalt sulphide, which are usually subjected to wet oxidation and then analysed. Preliminary experiments have indicated that the hydriodic acid treatment gives satisfactory results for the determination of sulphur contents of the sulphides of silver, nickel and cobalt. This method will not, however, give values for free sulphur or sulphates, since sulphates are not usually reduced

in dilute solution. This method cannot be applied to the analysis of pyrites and chalcopyrites, as the reaction between the reagent and the minerals is extremely slow.

We thank Professor K. R. Krishnaswami for his keen interest in the work.

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November 7th, 1955

THE FLUORINE CONTENT OF FLOUR AND CRETA

Samples are submitted to this Laboratory with reference to the provisions of the Flour Order,[1] and, as we had received enquiries about the fluorine content of flour, an opportunity was taken to

Table I

Creta and fluorine contents of bulked flour samples for months of January, February and March 1955

Flour type	Mill group	Creta content, oz per sack	Fluorine content, p.p.m.
LE	A (under 5 sacks per hour)	12	0·8
LE	B (6 to 10 sacks per hour)	11	0·7
LE	C (11 to 20 sacks per hour)	11	0·8
LE	D (21 to 50 sacks per hour)	12	0·7
LE	E (over 50 sacks per hour)	13	0·7
IL	All	11	0·6
NS	A	14	0·9
NS	B	11	0·8
NS	C	14	1·0
NS	D	13	0·8
NS	E	15	1·0
ND	A	14	0·8
ND	B	14	0·8
ND	C	13	0·8
ND	D	13	0·8
ND	E	12	0·9
IN	All	12	0·5
BS < 93	All	10	0·6
BS > 93	All	9	0·7
BD	All	10	0·6
Art. 3 (*ii*)	All	1	0·3
Creta from supplier X	—	—	225
Creta from supplier Y	—	—	230

Key—

Home produced flours—

LE = low extraction, *i.e.*, less than 80 per cent. extraction.
NS = National Straight Run, declared 80 per cent. extraction.
ND = National by divide process, simulated 80 per cent. extraction.
BS < 93 = National Brown Straight Run, less than 93 per cent. extraction.
BS > 93 = National Brown Straight Run, greater than 93 per cent. extraction.
BD = National Brown by divide process.

Art. 3 (*ii*) = 100 per cent. extraction wholemeal, creta addition not compulsory.

Imported flours—

IL = low extraction, *i.e.*, less than 80 per cent. extraction.
IN = National, declared 80 per cent. extraction.
(Note—No verification of the declared extraction rate is implied by the authors.)

establish the content of representative samples. The values obtained, shown in Table I, may be of interest. The procedure of the Society's Analytical Methods Committee[2] was employed. The representative samples were obtained by bulking all samples that were received during the first quarter of 1955, so that each bulk sample represented a particular type of flour. Bulk samples of creta from the two main suppliers were prepared in a similar way. The mill classification is that instituted by the Ministry of Food, wherein mills are grouped according to their output capacity.

Creta is compulsorily added to all flour, except that of 100 per cent. extraction, at the rate of 14 oz per sack (0·31 per cent.). It will be seen that this supplement will contribute 0·7 p.p.m. of fluorine to flour when the creta contains 230 p.p.m. of fluorine, and that the fluorine content of these bulk samples examined is mainly attributable to the creta addition.

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February 20th, 1956

AN IMPROVED SPOT TEST FOR THE DETECTION OF ASCORBIC ACID IN FLOUR

With the recent introduction of ascorbic acid as a commercial flour improver, the need has arisen for a rapid routine test for its detection in bread flours. Several tests have been examined for specificity and sensitivity in the range 10 to 20 p.p.m. of ascorbic acid, and a method applicable in the presence of other flour additives has been devised.

Hitherto, the detection of small quantities of ascorbic acid in foodstuffs and the like has been achieved by the qualitative application of Tillman's 2:6-dichlorophenolindophenol reagent. Treatment of a flour sample with a dilute aqueous solution of this reagent reveals the presence of ascorbic acid as light spots on a blue or purplish background. The test may be misleading if the particles of ascorbic acid are very small or if the flour contains active benzoyl peroxide. Comparatively rapid deterioration of the reagent solution is a further disadvantage.

Experimental

Many of the known colour reactions of ascorbic acid were briefly investigated, but the majority of these were rejected owing to lack of sensitivity or simplicity of application. The most promising results were obtained with Tauber's ferric - ferricyanide reagent.[1] In accordance with the original details of the method, adequate response was shown at very low levels of ascorbic acid, a positive test being indicated by the formation of bright blue spots on a yellow background. The smallest particles of ascorbic acid were identifiable with this reagent.

In the examination of a number of commercial flours, however, it was found that blue spots often appeared when it was known that ascorbic acid treatment had not been used. The spots appeared more slowly than those characteristic of ascorbic acid, and showed greater uniformity of shape and size. These results were attributed to the presence of reduced iron added to the flour in the form of enrichment mix.

Modification of Tauber's reagent to reduce the total acidity and thus lower the tendency for ferrous iron to pass into solution successfully eliminated this interference. The modified reagent and procedure as described below was capable of detecting 10 p.p.m. or less of ascorbic acid, and was unaffected by all the commonly used improvers and enrichment materials.

Method

Reagents—

Modified Tauber reagent—Dissolve 1 g of ferrous sulphate in 50 ml of water and add 10 ml of 85 per cent. phosphoric acid. Heat to boiling, and oxidise by adding a 1 per cent. solution of potassium permanganate until a faint pink colour persists. Cool, and add 20 per cent. sodium hydroxide solution until a permanent turbidity is produced. Clear the solution by cautious addition of 10 per cent. sulphuric acid, and then make up to 100 ml and filter. Add 0·5 g of potassium ferricyanide dissolved in 100 ml of water and mix.

The mixed reagent should be pure yellow in colour and remains active so long as no change in colour occurs. In this laboratory the reagent has been stored for periods up to 3 months without loss of sensitivity.

PROCEDURE—

Smooth a sample of the flour on a white tile or Pekar slab and wet it by quickly immersing the tile or slab in water and draining. Treat the wet surface with sufficient of the modified Tauber reagent to cover completely and leave for 1 to 2 minutes. The presence of ascorbic acid is indicated by the appearance of bright blue spots or flecks. The level of treatment may be approximated by comparison with flours containing known amounts of ascorbic acid.

It is important that the moistened test sample should be examined for the formation of spots in the time specified, as, in exceptional cases, a slow response may occur as a result of treatment with reduced iron. Such a response is unlikely in the absence of excessive chlorine treatment, which may render the iron particles more reactive, but, should the necessity arise, closer examination of the spots by means of a hand lens will reveal their origin. Spots due to ascorbic acid are irregular in shape and size, whereas those due to reduced iron appear as blue points surrounded by a diffuse ring.

I thank the Directors of Novadel Ltd. for permission to publish this Note.

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NOVADEL LIMITED
ST. ANN'S CRESCENT
LONDON, S.W.18

K. J. HAYDEN
January 5th, 1956

Apparatus

IMPROVED SAMPLING APPARATUS FOR WATER CONTAINING DISSOLVED OXYGEN

FOR experiments made in this laboratory, pairs of identical samples of river water were required as a starting point. A conventional sampling canister was adapted to fill two bottles in series, but it was found that the samples gave different values for dissolved oxygen. The search for a simple means of taking two identical samples has disclosed faults that can arise in the use of a sampling canister, so that errors hitherto disregarded as trivial may be introduced into the Winkler method of determining dissolved oxygen.

The sampling canister used in the laboratory is of conventional type and contains a bottle of about 500 ml capacity. The inlet tube reaches down to the bottom of the bottle, and river water first fills the bottle and then overflows into the canister, which is of sufficient capacity to receive more than four times the amount of water in the bottle. Air is displaced through a tube in the lid of the canister to which a six-foot length of rubber tubing is attached: the tubing performs the triple function of leading air to the surface, acting as a depth gauge and indicating when the canister is filled completely.

For our special experiments the canister was adapted to take duplicate samples by connecting two smaller bottles in series so that the water swept out the first bottle, then the second bottle and finally filled the canister.

Samples taken in this manner showed that the contents of the second bottle were invariably more oxidised than those in the first bottle, equivalent to a gain of 0·15 to 0·24 p.p.m. of dissolved oxygen. Observation showed that this was caused by water in the inlet tube, some 50 to 60 ml, that had run back into the canister as it was raised out of the river. This water had become aerated in the tubing and, in running back, some of it had found its way into the second bottle, in turn displacing a similar quantity of water into the first bottle and giving a final displacement through what is normally the inlet of the apparatus. The improved apparatus is shown in Fig. 1. An enema-tubing valve, A, was inserted in the bottom of the outlet tubing to hold up the water column; it was most effective and there was no leakage back. As an additional precaution (although hardly necessary), a stopper was placed in the top of the tubing after the sample had been taken.

Duplicate samples gave much better agreement after the non-return valve was fitted, but now showed a small but definite oxidation in the first bottle, equivalent to 0·035 to 0·065 p.p.m. of dissolved oxygen. This was shown to be due to the presence of a small quantity of air introduced into the first bottle by carrying the apparatus from the boat to the laboratory. The system

was effectively sealed by placing a stopper in the inlet tube, B, immediately the canister was raised from the river. Later, a canister with dual inlets was made with small taps fitted close to the lid to seal off the contents after the sample has been taken.

A further improvement was added at this stage. A short length of glass tubing was inserted in the rubber tubing from the outlet of the apparatus to give a window, C, about 2 to 3 inches long, the mid-point of which was just 6 feet above the inlet of the canister. This acted as a depth

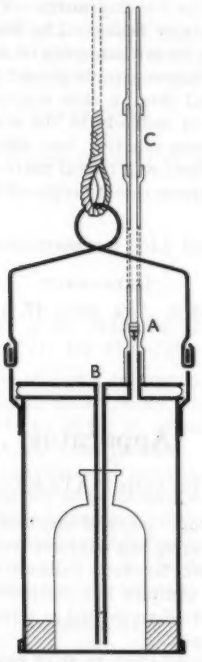

Fig. 1. Sampling apparatus: A, enema-tubing valve; B, point of sealing inlet; C, glass window

gauge, as a signal that the canister had filled and permitted air to escape freely above the water surface with no possibility of water entering the outlet to impede the rate of filling, even in choppy water.

Pairs of samples taken in a canister fitted with these improvements gave satisfactory agreement within experimental error and the small differences found (average, 0·012 p.p.m. of dissolved oxygen) were not all in the same direction.

All apparatus used in this laboratory for taking single or dual samples has been treated in this manner and has given satisfactory and trouble-free service. It has been particularly valuable for the collection of samples that contain sulphide and have to be treated in the complete absence of oxygen. Such samples have been treated by a modification of the Winkler method, in which sulphide is measured by absorption of iodine.[1] This has permitted us to determine as a routine either total sulphide content, or dissolved oxygen and sulphide sulmultaneously,[2] or to apply the appropriate correction in dissolved oxygen determinations for the presence of sulphide in river-water solids.

We thank Dr. J. A. Scott, Medical Officer, and Dr. S. G. Burgess, Scientific Adviser, for permission to publish this work.

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1. Houlihan, J. E., and Farina, P. E. L., *Sewage Industr. Wastes*, 1952, **24**, 157.
2. ——, ——, unpublished work.

SCIENTIFIC BRANCH, PUBLIC HEALTH DEPARTMENT
L.C.C. SOUTHERN OUTFALL WORKS
CROSSNESS, ABBEY WOOD, S.E.2

J. E. HOULIHAN
P. E. L. FARINA

October 13th, 1956

British Standards Institution

NEW SPECIFICATIONS*

B.S. 2734: 1956. Boiling Flasks (Narrow-necked): Conical, Flat Bottom and Round Bottom. Price 2s. 6d.

B.S. 2736: 1956. Reference Thermometers for Field Use. Price 2s. 6d.

AMENDMENT SLIPS*

PRINTED slips bearing amendments to British Standards have been issued by the Institution, as follows—

PD 2446—Amendment No. 1 (April, 1956) to B.S. 2533: 1954. Chlorobenzene.
PD 2461—Amendment No. 1 (April, 1956) to B.S. 795: 1953. Ampoules.

Book Review

NEW METHODS IN ANALYTICAL CHEMISTRY. By R. BELCHER, B.Sc., Ph.D., F.R.I.C., F.Inst.F., and C. L. WILSON, Ph.D., D.Sc., F.R.I.C., F.I.C.I. Pp. xii + 287. London: Chapman & Hall Ltd. 1955. Price 30s.

The title of this book may well be misleading to the unwary for the book deals not with modern physical or instrumental methods of chemical analysis, as might well be expected from its title, but with much that is new or comparatively new in the orthodox fields of gravimetric and volumetric analysis. It is, indeed, a compilation of methods, selected from the literature of the last twenty years or so, that have not, in the main, found their way into the standard textbooks of classical methods of analysis. Some of the methods are too recent in origin so to be included, others have not been sufficiently tested to merit confidence, whereas others, like the silver-reductor method for determining iron, are only just beginning to be included after too long a period of neglect. First-class methods of analysis are often few and far between and a cautious approach to new methods by authors of standard textbooks is more than justified.

This book is divided into seven chapters, the first of which deals with the use of novel precipitants, such as nitric acid for separating strontium from calcium, or with the use of novel methods of precipitation, such as the controlled precipitation of certain basic formates by the hydrolysis of urea, or of certain oxalates and phosphates by the hydrolysis of methyl oxalate and trimethyl phosphate, respectively. In the description of the precipitation of strontium, barium and lead as nitrates, however, the authors have not made it clear that the concentrated nitric acid to which they refer is not the 70 per cent. acid common in the laboratory, but the 100 per cent. acid, which the original authors of the method were careful to specify. An analyst trying out the methods might easily be confused and misled.

In Chapter II, procedures that depend on the extraction of inorganic salts and organo-metallic complexes by organic solvents are described, whilst in Chapter III, inorganic precipitants such as iodic acid, periodic acid and potassium cobalticyanide are described in their applications to determinations of several cations. Precipitants such as *trans*-dichlorobisethylenediaminecobalt(III) chloride, a reagent for quinquevalent antimony, are included in more selective applications.

In Chapter IV, the uses of twenty-four organic reagents ranging from alizarin blue as a precipitant for cupric ions, through ethylenediaminetetra-acetic acid in some of its numerous applications, to triphenylmethylarsonium iodide as a reagent for the gravimetric determination of cadmium in the presence of zinc are all discussed, and details of procedure are given.

Indicators of various types are dealt with in Chapter V, where the information given should interest many analysts—especially as many of the reagents can be easily acquired—and encourage them to make wider use of some of the indicators mentioned. Screened indicators, mixed indicators and redox indicators, such as 1:10-phenanthroline and N-methyldiphenylamine-*p*-sulphonic acid, a particularly good indicator in my opinion, ought to be more popular than they are.

* Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

In Chapter VI, the titrants sodium chlorite, potassium iodate, calcium hypochlorite, mercurous nitrate, stannous chloride, potassium diperiodatocuprate and ethylenediaminetetra-acetic acid receive attention, the last in its now well known application to the determination of calcium and magnesium hardness in water. One wonders whether the section on stannous chloride as a titrant is worth its place in this chapter. Few analysts would make this reagent their first choice and the substances to the determination of which it is applied can surely be determined equally well and probably better by other means.

The last and much the longest chapter gives us a collection of miscellaneous methods, twenty-four in all. Of these, methods for potassium as potassium tetraphenylboron, ferric iron by the silver reductor, phosphate and silicate based on the formation of quinoline phospho- and silico-molybdates, respectively, together with methods for calcium and for germanium, seem to be the most interesting. Hoffman and Lundell's procedure (*J. Res. Nat. Bur. Stand.*, **1937**, **19**, 59) for determining P_2O_5 in phosphate rock gravimetrically by direct precipitation with magnesia mixture in the presence of a high concentration of citrate ions, a preliminary precipitation by ammonium molybdate thus being eliminated, might well have been included in this chapter. It is good to see mentioned the silver-reductor method for iron, but a fuller description is necessary before recourse to the original papers can be avoided. Further, it is a pity to revive a method involving the weighing of calcium oxalate as the monohydrate, with its attendant uncertainties, when the conversion of oxalate to carbonate can, in the easily controllable electric furnaces now available, be so readily accomplished.

Other points call for comment in a review. The first reference given on p. 124 is wrong; it should be M. Venkataramaniah and B. S. V. Rao, *Analyst*, **1950**, **75**, 553. There is a slip in the first equation on p. 181, where $2H_2O$ should be $3H_2O$ and another on p. 119, line 8, where "weighing" should be "weighting," which makes sense where "weighing" does not. Four of the conversion factors on pp. 178 and 179, and the one on p. 182, are wrong, and a humorous touch would be lost had the authors not written as they did on p. 214, "When dried at 105° C the results are closer to theory. . . ." Finally, their habit of omitting the word "of" in the detailed descriptions of procedures that they give leads to such infelicities as "Add a few grams ground sodium hydroxide . . .", p. 93, and, p. 88, "a few drops acetone."

The book is a successor to one published in 1932 by Dr. A. D. Mitchell and Dr. A. M. Ward and, like its predecessor, aims at bringing to the notice of analysts methods that may be of use to them in their work. In this it should be successful and serve a useful purpose. One of the many merits of Mitchell and Ward's book was that most, if not all, of the methods described were tried out by them before being recommended to the reader. This has been done, the preface tells us, with some of the methods now given—they are too numerous for it to have been done to all—but, unfortunately, Dr. Belcher and Dr. Wilson do not tell us which these are. Their book would have been of greater value had they done so.

Let us join the authors in hoping that many analysts will be encouraged by the publication of this book to try out for themselves some of the methods given, for it is only by action of this kind that really first-class and trustworthy methods of analysis can be sorted out and given their due.

L. S. THEOBALD

Publications Received

THE SYSTEMATIC IDENTIFICATION OF ORGANIC COMPOUNDS. By RALPH L. SHRINER, REYNOLD C. FUSON and DAVID Y. CURTIN. Fourth Edition. Pp. x + 426. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. Price $6.00; 48s.

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FOOD POISONING. By G. M. DACK, Ph.D., M.D. Third Edition. Pp. xii + 251. University of Chicago Press; Cambridge University Press. 1956. Price 45s.

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